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THE BORON DEFICIENCY DISEASE IN CABBAGE [1]

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INTRODUCTION

As early as 1900, Copeland and Kahlenberg (*9*)[2] pointed out that the root development of lupine (*Lupinus albus* L.) seedlings seemed to be stimulated when a trace of boron was added to the water in which they were growing. Three years later Nakamura (*20*) reported stimulation of growth of peas (*Pisum sativum* L.) when borax was added to the soil at the rate of 1 mg. per kilogram. Agulhon (*1*) pointed out in 1910 that boron was a useful element to many plants, including radish (*Raphanus sativus* L.) and turnip (*Brassica rapa* L.). At about the same time in the Philippine Islands, Roxas (*23*), at the suggestion of Copeland, studied the effect of applying borax to potted soil in which rice (*Oryza sativa* L.) was planted. Although toxic effects resulted at some concentrations, it was observed that one application of M/100,000 boron stimulated leaf growth. In 1914 Brenchley (*5*) pointed out that in liquid-nutrient cultures the growth of peas was stimulated by certain very low concentrations of boron as boric acid, and in 1916 Moore (*3*) reported similar results with radish in both liquid- and sand-nutrient cultures.

Mazé (*17, 18*) in 1919 offered the first proof that traces of boron were necessary for the growth of corn (*Zea mays* L.). In 1923 Warington (*26*), working in Brenchley's laboratory, concluded that certain species of legumes require boron for normal development and that the absence or deficiency of this element in the nutrient solution brought about definite pathological symptoms. She did not prove it to be absolutely essential for barley or rye. At the present time it is rather generally accepted that boron is an essential minor element for most, if not all, higher plants and, furthermore, that certain species require relatively more than others for their normal development.

In 1930 Mes (*19*) pointed out that the symptoms of boron deficiency produced on tobacco in nutrient solution were similar to the top rot disease in Sumatra, and in the following year Brandenburg (*4*) offered proof that the long-recognized heart rot and dry rot of sugar beet (*Beta vulgaris* L.) is a boron-deficiency disease. After these two discoveries, numerous diseases of many crops were rapidly shown to be due to boron deficiency. In this list are various diseases of crucifers. MacLeod and Howatt (*16*), working in eastern Canada and reporting in 1935, were the first to show that brown heart of rutabaga (*Brassica campestris* var. *napobrassica* DC.) and turnip

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[2] Italic numbers in parentheses refer to Literature Cited, p. 586.

was reduced and often prevented entirely in the field by the application of borax to the soil. This result was confirmed during the same year in Wales (*27*), in Finland (*14*), and in Scotland (*22*). It was also in 1935 that the internal and external browning of the curd of cauliflower (*Brassica oleracea* var. *botrytis* L.) in the Catskill Mountain district of New York State was shown to be a boron-deficiency disease by Dearborn et al. (*10, 11*). This was confirmed by Hartman (*13*) on Long Island in 1936, by Chandler et al. (*7*) in Maine in 1937, and by Ferguson (*12*) in Canada in 1938. Hartman (*13*) also pointed out that cabbage (*B. oleracea* var. *capitata* L.) growing adjacent to severely affected cauliflower produced large marketable heads. Chandler et al. (*6, 8*) reported further that water-soaked brown areas developed in the fleshy stems of boron-deficient kohlrabi (*B. oleracea* var. *gongylodes* L.) and kale (*B. oleracea* var. *viridis* L.), while in sprouting broccoli (*B. oleracea* var. *botrytis* L.) abscission of buds occurred. Symptoms of boron-deficiency were observed in the field, however, only in cauliflower and turnip. When acute shortage of boron prevailed, as provided in controlled nutrient culture, the formation of storage organs of the cole group was suppressed or prevented.

The purpose of the present paper is to report experiments, conducted in both greenhouse and field, upon the internal break-down of cabbage and the closely related plants, kale, collard (*B. oleracea* var. *viridis* L.), and sprouting broccoli when boron deficiency in the soil occurs. Interest in this problem arose in connection with the internal break-down of cabbage heads affected with a mosaic disease (*15*).

METHODS AND MATERIALS

Plants were grown in the greenhouse at Madison, Wis., in sand cultures provided with a constant supply of nutrient by a modification of the method described by Allison and Shive (*2*). Clean 8-inch clay pots were varnished, provided with adequate drains, and filled with washed white quartz sand. The flow of nutrient was adjusted to deliver about 1 liter per day to each pot. Shive's Best three-salt solution [3] (*25*), to which traces of copper as sulfate ($CuSO_4.5H_2O$), iron as sulfate ($FeSO_4.7H_2O$), zinc as sulfate ($ZnSO_4.7H_2O$), and manganese as chloride ($MnCl_2.4H_2O$) had been added, was diluted 10 times with distilled water to provide a basic nutrient solution with a total salt concentration of 0.738 gm. per liter. This is referred to as the boron-free nutrient solution. Care was taken to avoid contamination with boron by using boron-free glassware. For the complete nutrient solution boric acid was added in amounts sufficient to provide 0.75 p. p. m. of boron. Seeds of cabbage (five varieties), turnip (two varieties), cauliflower, rutabaga, radish, and kohlrabi were sown directly in the pots. When plants were transferred from complete to boron-free nutrient and vice versa, first all were removed; some were transplanted back into the original pots, others were exchanged and transplanted. In this way both control and exchanged plants were comparable insofar as handling was concerned.

Field studies were carried out near Winneconne, Winnebago County, Wis., on Poygan silty clay loam with a soil reaction of about pH 7.5. The field had had an application of barnyard manure and 2 tons of

[3] The formula for this solution is: Monopotassium phosphate (KH_2PO_4), 0.018 M; calcium nitrate ($Ca(NO_3)_2.4H_2O$), 0.0052 M; and magnesium sulfate ($MgSO_4.7H_2O$), 0.0150 M.

ground limestone per acre in the spring of 1936. In 1937 a crop of sugar beets grown on it had shown severe heart rot. The field experiments reported herein were conducted on this area in 1938, 1939, and 1940.

Plots 30 by 30 feet, with 5-foot borders, were laid out in 4 blocks of 9 treatments each, and the arrangement of the treatments was randomized within each block. Borax and salts of the other minor elements used were applied broadcast with the fertilizer by means of a lawn fertilizer drill, and harrowed thoroughly into the soil 5 weeks before the crops were planted.

EXPERIMENTAL RESULTS

SYMPTOMS ON SAND-NUTRIENT-CULTURE PLANTS

The expression of boron-deficiency symptoms on cabbage varies with the age of the plant and with the extent to which it is deprived of this element. When seedlings are grown in sand-nutrient culture from which boron is excluded, the disease develops rapidly and growth is sharply repressed. In soil in the greenhouse or in the field, boron starvation is neither so rapid nor so complete.

Symptoms of boron deficiency were produced experimentally on sand-nutrient-culture plants in the greenhouse. They will be described first. The field symptoms will then be discussed in comparison with those on cauliflower, kale, collard, and sprouting broccoli.

In sand-nutrient cultures, cabbage seedlings emerged 5 to 7 days after the seed was sown. When a boron-free nutrient solution was supplied the first effects were apparent in 7 to 10 days after emergence, usually most conspicuously on the first leaf, for the cotyledons as a rule expanded quite normally. The leaf blade became linear or circular in shape (fig. 1, *A* and *B*). Lateral venation was reduced and sometimes was almost entirely absent, while the leaf lamina may have split away from the midrib. The affected leaf was usually abnormally dark green and was somewhat thicker than the normal leaf. Early death of the growing point commonly occurred (fig. 1, *A* and *B*), and as growth of buds at the cotyledonary and other leaf nodes followed, the stunting of the plant was even more pronounced (fig. 2, *A*).

When plants started in a boron-free nutrient solution were transferred to a complete solution, rather prompt recovery occurred. New growth frequently took place from an axillary bud, as shown in figure 2, *C*. If, however, the growing point had not been killed new leaves formed normally. When seedling plants growing in a complete solution were transferred to a boron-free nutrient solution, the effects of the deficiency were apparent in 2 to 7 days. The leaves which had reached their full size were not affected, but the young unfolding ones at once began to assume the distorted morphology found in plants that start off in the deficient solution (fig. 2, *B*). Seedlings of cauliflower, kohlrabi, rutabaga, turnip, and icicle radish responded very similarly to cabbage when they were grown in boron-free nutrient solution.

The symptoms described above are those resulting when conditions for complete boron starvation of young seedlings are provided. When plants of cabbage are allowed to grow to greater size in a complete nutrient solution and the boron supply is then greatly reduced without

being completely excluded, leaf malformation is again the chief sign of deficiency but internal break-down of the pith may also occur. Variation between individuals in the expression of abnormal growth is more noticeable in the somewhat older plants than in young seedlings. In figure 3 the leaves from plants grown in a boron-deficient nutrient solution are compared with those on plants of the same age grown continuously in the complete solution. The plants were started from seed in complete nutrient solution. After 17 days the boron supply was reduced to a very slight trace for some plants. Signs of boron deficiency appeared in 10 days, and although the growth rate was not materially reduced, various manifestations con-

FIGURE 1.—Cabbage seedlings (Jersey Queen variety) grown from seed in sand-nutrient culture for 31 days: *A* and *B*, grown in boron-free nutrient solution; *C*, grown in complete nutrient solution. Note that the cotyledons are normal in all three, but in the boron-deficient plants the growing points are killed and the leaves are dwarfed and distorted.

tinued to develop during several weeks. One of the signs of boron deficiency is a darker green color of one or more leaves; later the plant may produce more normally colored leaves. Puckering of the leaf blade in various ways, but more commonly near the midrib, may follow (fig. 3, *B*, *C*). This distortion is the result of one or both of two causes: (1) The retardation of growth of the tissue near the margin of the leaf and (2) stimulation of excess growth in the region of the midrib not unlike that discussed by Van Schreven (*24*). If the lack of boron affects the leaf earlier in its development, linear formation accompanied by curling from the suppression of marginal growth follows (*C*, *F*); or even greater stunting and malformation may result in lateral distortion or suppressed lateral venation with segments of the leaf entirely absent (*D*, *E*). Occasionally cross hatching of the upper surface of the midrib occurs (*G*), resembling that commonly associated with boron deficiency in sugar beet (*4*). An interveinal marginal yellowing sometimes appears on the older leaves of boron-

deficient plants (*H*, *I*) after symptoms of malformation have appeared. Yellowing may appear later on the younger leaves.

SYMPTOMS ON FIELD-GROWN PLANTS

It should be emphasized that it was commonly the case that no external symptoms of boron deficiency in cabbage were seen in the field even when the crop was grown on soil where sugar beet, table beet, and cauliflower showed external signs. This is in accord with the observations of Hartman (*13*), cited above. The field in which

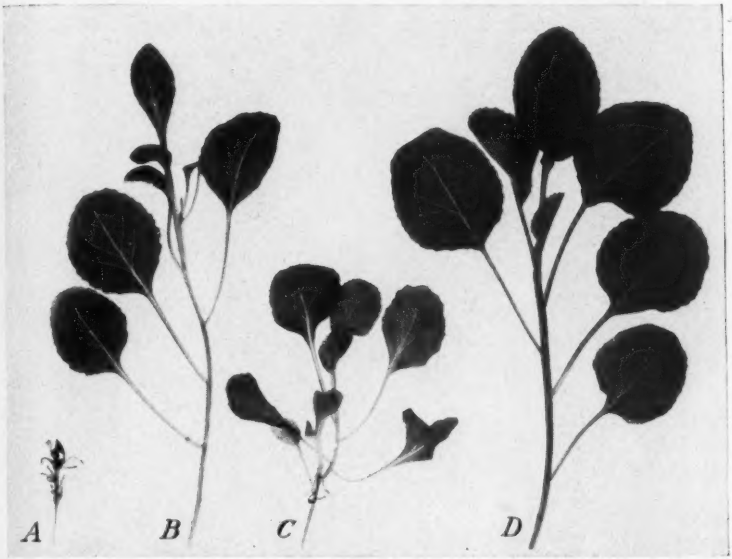

FIGURE 2.—Cabbage seedlings (Jersey Queen variety) grown in sand-nutrient culture: *A*, Grown for 73 days from seed in boron-free nutrient solution; *B*, grown in complete nutrient solution for 33 days, then transplanted to boron-free culture and continued for 40 days (note distortion of the youngest leaves); *C*, grown for 33 days in boron-free nutrient solution, then transplanted to complete solution and continued for 40 days (note that the growing point was killed and that the new growth proceeded normally from a leaf node); *D*, grown in complete solution for 73 days.

the following observations were made was one in which heart rot of sugar beet was severe in 1939. The appearance of cauliflower in midseason in that year is shown in figure 4. No external symptoms were found in cabbage in 1939, and the size, solidity, and weight of cabbage heads were not noticeably different in plants grown on this soil from those grown on the same soil where 40 pounds of borax per acre had been applied, a treatment that corrected the disease in sugar beet and cauliflower. In 1940 cabbage transplanted to these plots in June showed in mid-August moderate symptoms on the outer leaves with occasional stunting in growth. The symptoms included mottling along the margins of the oldest leaves, cross hatching on the upper surface of the petiole and midrib, abnormal thickening

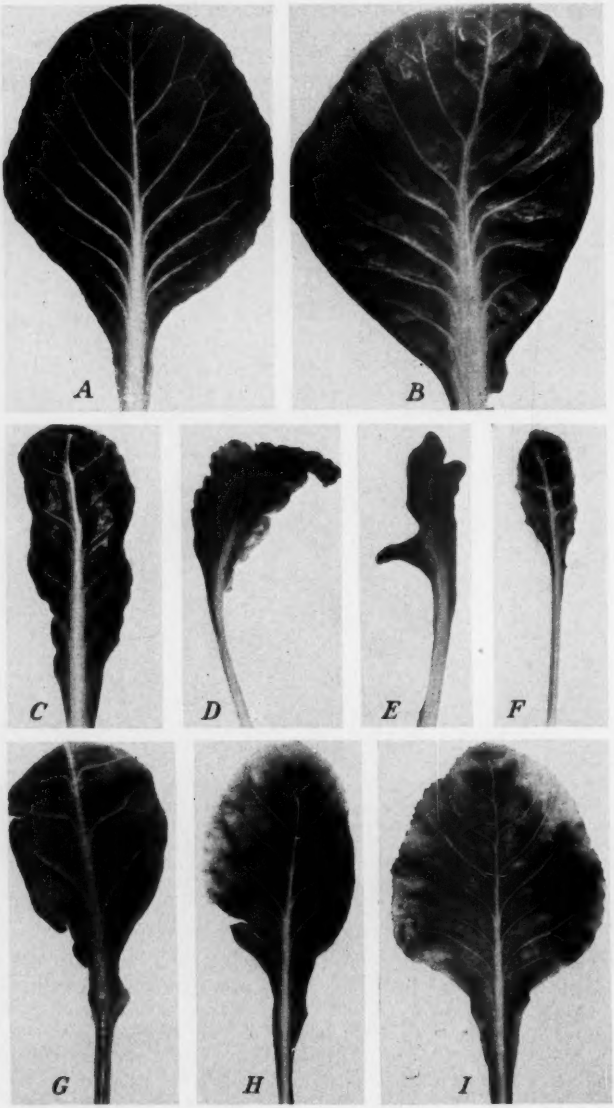

FIGURE 3.—*A*, Leaf from plant grown in complete solution; *B* to *I*, leaves from plants grown with a reduced boron supply. Photographed when plants were 120 to 130 days old. (See further explanation in text.)

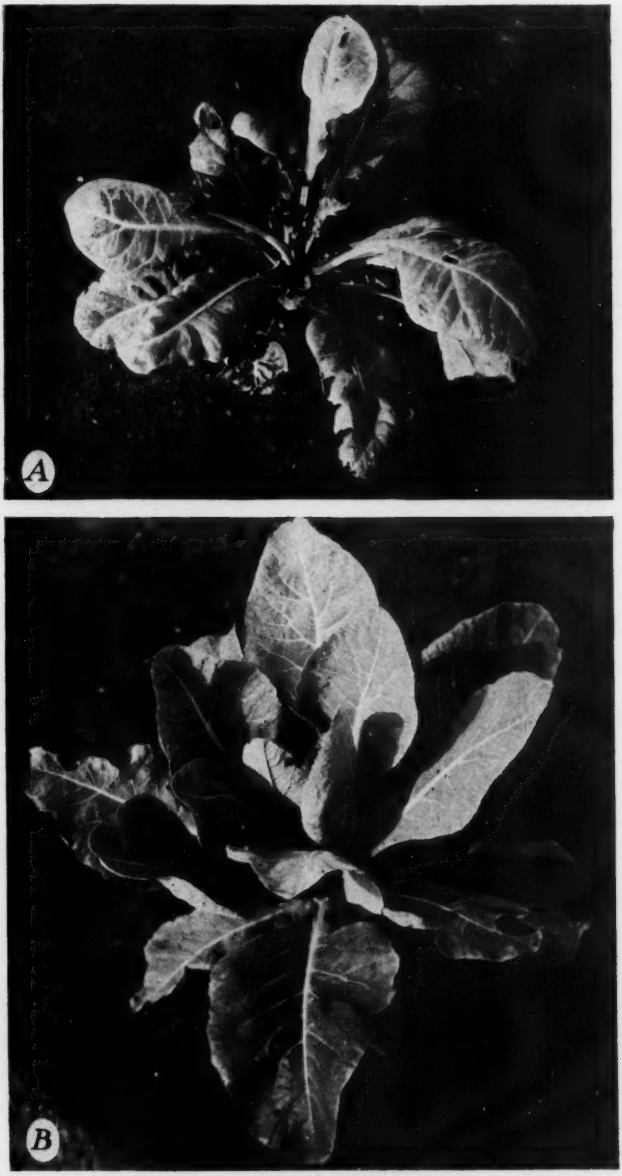

FIGURE 4.—*A*, Cauliflower grown on boron-deficient soil; *B*, comparable plant grown on an adjoining plot to which 40 pounds of borax per acre was applied before planting. Note reduced size of diseased plant (*A*), downward marginal curl of oldest leaves resulting from suppressed marginal growth, and reduced size and distortion of the youngest leaves.

of the leaf lamina, unilateral leaf development, and curling of the leaf margin. Younger leaves usually developed without symptoms.

When mature cabbage plants grown on boron-deficient soil were examined by making a vertical cut through the head so as to bisect the main stem longitudinally, the pathological symptoms were found in the pith of the stem. They were not unlike those described by Dearborn et al. (*10*, *11*) in cauliflower stems. Water-soaked spots developed first, and the tissue therein gradually turned brown while the lesions enlarged. Necrotic spots thus formed, usually midway between the top of the core and the base of the head (fig. 5), but often extending well into the lower stem. Sometimes the break-down increased to a point where a cavity formed (fig. 6). This symptom, however, is relatively rare in cabbage under the same conditions in which it is common in cauliflower. No necrosis that can be attributed to boron deficiency has been noted in the leafy portion of cabbage heads except in the midrib adjacent to the main stem. The break-down in the core is of definite economic importance, particularly in the manufacture of sauerkraut, where the shredded pith is used as well as the leafy portion and where the discolored, corky, necrotic tissue detracts from the quality of the final product. Similar internal necrotic areas occurred in the field on kale, collard, and sprouting broccoli. The symptoms described are in general accord with those reported recently from Maine by Chandler (*6*).

RELATIVE SUSCEPTIBILITY OF SUBSPECIES OF BRASSICA OLERACEA

Seed of several members of the cole group was sown in the boron-deficient soil at Winneconne on May 25, 1938. The 30-foot single-row plots of each lot were placed in random arrangement in each of three blocks. The plants were thinned to 2-foot intervals on June 20, leaving 15 plants per row plot. On August 31 all cauliflower plants were sectioned and examined; the other subspecies were so handled on September 24. The varieties used and the results secured are given in table 1.

TABLE 1.—*Development of internal break-down in subspecies of Brassica oleracea grown on boron-deficient soil at Winneconne, Wis., 1938*

[15 plants used in each test]

Subspecies and variety	Plants showing internal break-down in—			
	Replicate 1	Replicate 2	Replicate 3	Average
	Percent	*Percent*	*Percent*	*Percent*
Cauliflower (Snowball)	93.3	93.3	73.3	86.6
Cabbage (Marion Market)	6.7	13.3	20.0	13.3
Broccoli (Green Sprouting)	0	33.3	0	11.1
Collard (Cabbage)	0	13.3	33.3	15.5
Kale (Thousand-headed)	46.7	20.0	20.0	28.9

While the differences between cabbage, broccoli, collard, and kale were not significant, it is very evident that even though they were allowed to stand approximately a month longer than the cauliflower, as a group they showed much less of the disease than the cauliflower. Moreover, the full extent of the difference is not expressed in percentage of plants affected, for the degree of break-down was also correspondingly greater in cauliflower than in the other forms.

RELATIVE SUSCEPTIBILITY OF CABBAGE VARIETIES

Several varieties of cabbage, representing a range from the early-maturing type, Golden Acre, to the late-storage form, Wisconsin

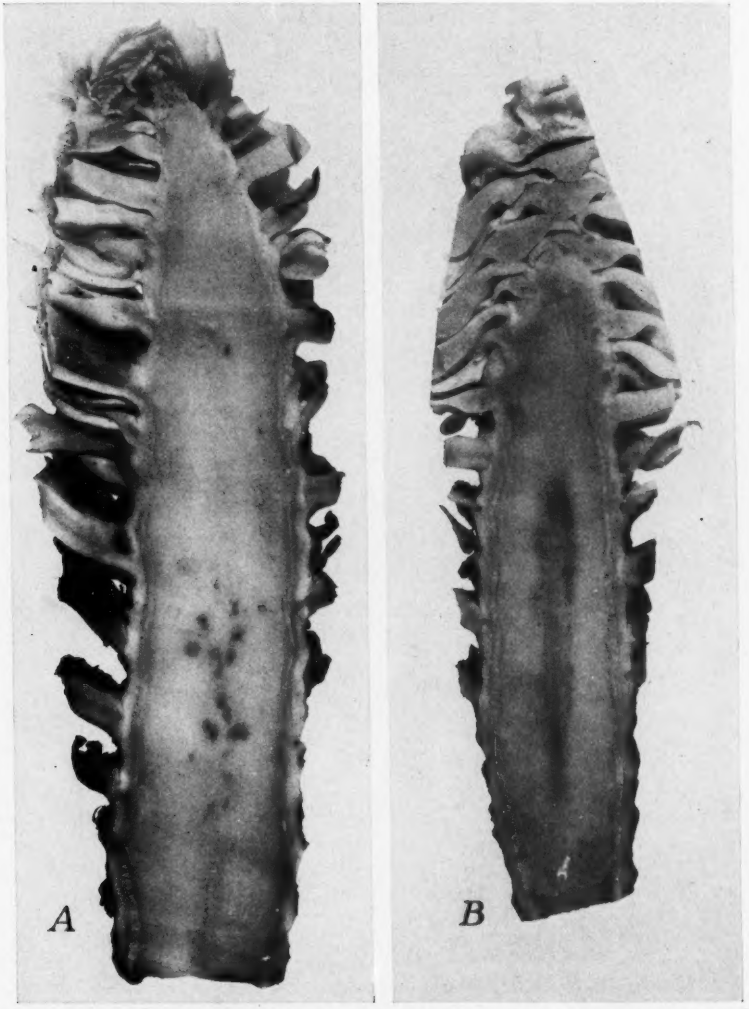

FIGURE 5.—Internal necrosis of stem of cabbage grown on boron-deficient soil: *A*, Scattered necrotic spots; *B*, a more extensive area with concentrated necrotic tissue.

Hollander, were transplanted to the Winneconne field in the latter part of June of 1939 and 1940. The seedlings had been grown in the seedbed at Madison on soil not known to be boron-deficient.

For each variety a single-row plot of 30 plants in 1939 and of 15 plants in 1940 was placed at random in each of 3 blocks. All plants grew without external symptoms in 1939, but in 1940 symptoms

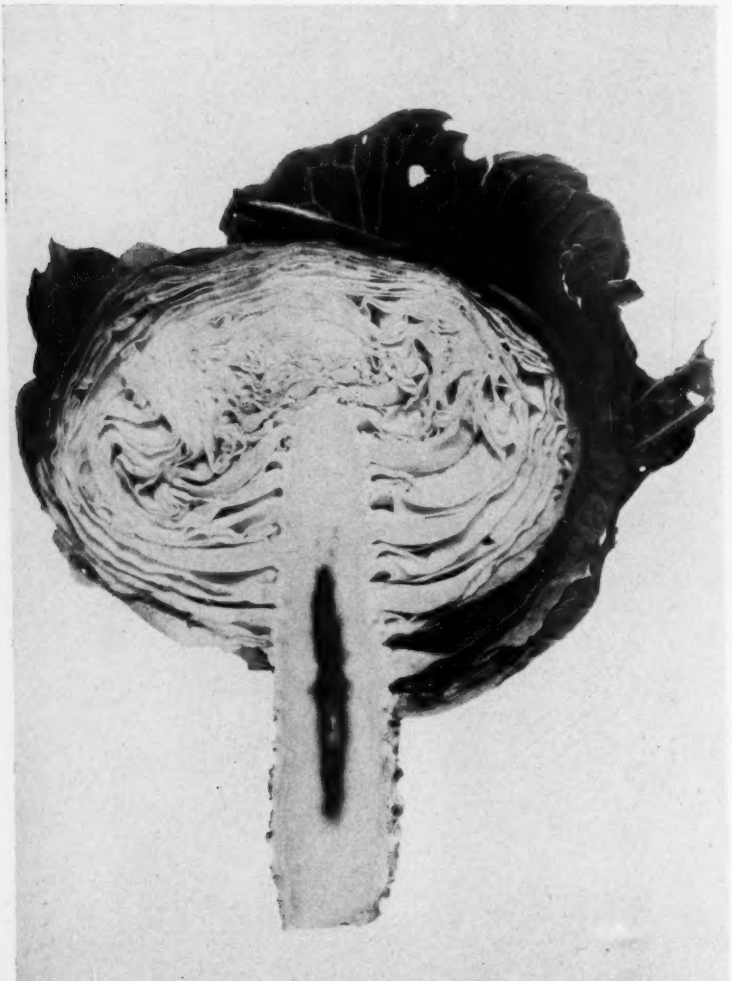

FIGURE 6.—Extreme stage of internal break-down of cabbage grown on boron-deficient soil.

already described occurred on the outer leaves of some plants. As the heads became mature they were sliced and the extent of internal break-down was recorded (table 2).

TABLE 2.—*Occurrence of internal break-down in several varieties of cabbage and in cauliflower grown on boron-deficient soil at Winneconne, Wis., 1939 and 1940*

[Average of 3 replicates in each year]

Subspecies and variety	1939		1940		
	Date of harvest	Internal break-down	Date of harvest	Internal break-down	Arc sin $\sqrt{x}$ radians
		Percent		*Percent*	
Cauliflower, Snowball			Sept. 19	98	1.4162
Cabbage:					
Jersey Queen			Oct. 7	6	.2505
Golden Acre	Sept. 14	67	do	60	.8870
Resistant Detroit	do	70	do	62	.9033
Racine Market			Oct. 17	43	.7126
Marion Market	Oct. 3	45	do	42	.7034
All Head Select	Oct. 26	26	do	15	.3993
Wisconsin All Seasons	do	36	do	10	.3158
Wisconsin Ballhead	do	33	do	33	.6134
Wisconsin Hollander	do	33	do	32	.6030
Resistant Red Hollander			do	58	.8705
Difference required for significance between varieties (19:1)		25			.3894

Of the white varieties, Golden Acre, Resistant Detroit, and Marion Market contained in both seasons the largest percentages of plants with internal break-down. In 1940 Racine Market was in this group. All four of these varieties are genetically related, since they were each derived by selection from Copenhagen Market. All Head Select, Wisconsin All Seasons, Wisconsin Ballhead, and Wisconsin Hollander were less seriously affected than the Copenhagen group.

The data in 1939 suggested that, since the earliest maturing varieties were the most seriously affected, a correlation between earliness and susceptibility might exist under these growing conditions. However, this does not hold in the 1940 data. Jersey Queen although of the same maturity as Golden Acre had significantly less disease than the latter, while Resistant Red Hollander of the same season as Wisconsin Hollander was about as seriously affected as Golden Acre. Cauliflower was distinctly more susceptible than all cabbage varieties tested.

CONTROL THROUGH SOIL TREATMENT

The proof that the internal break-down of cabbage was the result of boron deficiency in the soil was amplified by soil-treatment experiments conducted at Winneconne in 1938 and 1939. As already indicated, four replicate blocks were laid out in which treatment plots were placed at random. In 1938, because of other experimental work under way, it was not possible to include cabbage in all of these. However, Wisconsin All Seasons cabbage was planted in each plot of two of the blocks and Snowball cauliflower in each plot of the other two blocks. The various treatments and the percentage of plants showing internal break-down in each plot are given in table 3. As a rule, the disease was less serious in cabbage than in cauliflower. The percentage of affected plants was reduced very greatly when 20 pounds of borax per acre was applied. In the case of cauliflower, the disease was eliminated in the 40-pound and 60-pound plots, but a small percentage of diseased plants of cabbage remained in these treatments. Manganese sulfate, applied separately, seemed to have

no effect on the correction of internal break-down, nor did it have any consistent effect when used to supplement borax. The other minor elements tested likewise had no perceptible effect upon the reduction of the disease.

TABLE 3.—*Effect of borax and salts of certain other minor elements on the occurrence of internal break-down of cabbage and cauliflower at Winneconne, Wis., 1938*

[15 plants used in each test]

Soil treatment			Internal break-down in—					
			Cabbage			Cauliflower		
Fertilizer (3–12–12) per acre	Borax per acre	Manganese sulfate per acre	Block 1	Block 2	Average	Block 1	Block 2	Average
Pounds	*Pounds*	*Pounds*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0	0	0	[1]	20.0	20.0	86.7	40.0	63.3
450	0	0	[1]	26.7	26.7	66.7	53.3	60.0
450	0	100	73.3	53.3	63.4	13.3	100.0	56.7
450	20	0	0	20.0	10.0	6.7	0	3.4
450	20	50	0	0	0	0	6.7	3.4
450	20	[2] 50	0	6.7	3.4	40.0	6.7	23.4
450	40	0	0	6.7	3.4	0	0	0
450	40	50	6.7	[1]	6.7	0	0	0
450	60	0	6.7	0	3.4	0	0	0

[1] No notes were taken on account of water damage.
[2] Also 50 pounds each per acre of copper sulfate, iron sulfate, and zinc sulfate, and 100 pounds per acre of sodium chloride.

In 1939 the same blocks were used without further treatment except for the application of 300 pounds per acre of 3–12–12 fertilizer to each plot but the one in each block to which no fertilizer had been applied in 1938. Two varieties of cabbage, Marion Market and Wisconsin Ballhead, were planted throughout. The results at the end of the season are given in table 4. The percentages of internal break-down were very similar to those recorded in 1938, confirming the indication that boron deficiency is the cause of the break-down. It is of interest to note that in this soil the boron still available from the treatment of the previous year was sufficient to correct the disease as effectively as it had in that year.

TABLE 4.—*Effect of borax and salts of certain other minor elements applied at Winneconne, Wis., in the spring of 1938, on the occurrence of internal break-down in the cabbage crop of 1939*

Soil treatment				Internal break-down in—		
Fertilizer (3–12–12) per acre—		Borax per acre, 1938	Manganese sulfate per acre, 1938	Marion Market	Wisconsin Ballhead	Average
1938	1939					
Pounds	*Pounds*	*Pounds*	*Pounds*	*Percent*	*Percent*	*Percent*
0	0	0	0	[1] 13.3	[1] 11.7	12.5
450	300	0	0	25.0	16.7	20.9
450	300	0	100	26.3	21.7	24.0
450	300	20	0	0	1.7	.9
450	300	20	50	3.3	3.3	3.3
450	300	20	[2] 50	1.7	1.7	1.7
450	300	40	0	3.3	0	1.7
450	300	40	50	0	0	0
450	300	60	0	1.7	0	.9

[1] Average of 4 replicates of 15 plants each.
[2] Also 50 pounds each per acre of copper sulfate, iron sulfate, and zinc sulfate, and 100 pounds per acre of sodium chloride.

DISCUSSION

Internal break-down of the tissue of cabbage heads is a factor that may detract greatly from the quality of the final product. Suboxidation in storage and transit has been shown by Nelson (*21*) to be a causal factor. More recently Larson and Walker (*15*) have associated symptoms very similar to those described by Nelson (*21*) with a mosaic disease. The present study adds another causal factor that may be responsible for an internal necrosis in the cabbage head.

It is of importance to note that the internal break-down in cabbage due to boron deficiency appears to occur largely in the pith of the main stem or core. In this regard it is distinct in location from the other two diseases mentioned. Furthermore, cabbage, as judged by the varieties tested, is much less susceptible than cauliflower. On the other hand, the usual lack of distinguishable external symptoms in the field places the disease among those that are not ordinarily detected until the product has reached the final culinary or processing stages.

The fact that varieties seem to differ in their response to boron deficiency in the soil would seem to justify further inquiry into this phase of the disease. It is also equally evident that such a genetic tendency should be kept under consideration in any program of improvement of this crop plant through breeding.

The disease appears to be readily corrected by the addition of borax to the soil. However, it is significant that, in the limited number of experiments reported, the complete elimination of the internal necrosis in cabbage was not accomplished even at 60-pound-per-acre applications, while the more susceptible cauliflower was completely healthy after a 40-pound-per-acre treatment. It remains to be determined whether the small amount of necrosis that persisted was due to other factors, or whether the cauliflower plant has properties that result in greater efficiency in the use of the boron salt applied to the soil.

SUMMARY

The symptoms of boron deficiency in young cabbage plants growing in sand-nutrient cultures in the greenhouse are described.

On boron-deficient soil the field-grown crop often showed no external signs of disease. Internal break-down of the pith was the chief symptom in the tissue.

Cauliflower was found to be much more subject to internal break-down on boron-deficient soil than cabbage, kale, collard, or sprouting broccoli.

Of several varieties of cabbage tested on boron-deficient soil the early-maturing ones, Golden Acre and Resistant Detroit, showed the greatest amount of internal break-down.

Applications of 20 pounds or more of borax per acre practically though not entirely eliminated the disease.

These applications of borax were equally effective when the second successive crop of cabbage was grown, without further treatment of the soil with borax.

Salts of manganese, copper, iron, zinc, and sodium applied to the soil had no effect upon the occurrence of internal break-down.

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A BLUE STAIN FUNGUS, CERATOSTOMELLA MONTIUM N. SP., AND SOME YEASTS ASSOCIATED WITH TWO SPECIES OF DENDROCTONUS[1]

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INTRODUCTION

Blue stain is a concomitant of the infestation of trees by certain bark beetles. Stains associated with some species of beetles have been found in previous investigations to be caused by species of *Ceratostomella*, which are carried into galleries mined by the attacking insects and grow in the cells of the sapwood (*3*, *11*, *13*, *15*, *16*).[3] It is believed that these fungi may be an important factor in causing the death of the trees. An investigation of the possible association of fungi with *Dendroctonus monticolae* Hopk. and *D. ponderosae* Hopk. resulted in finding a new species of *Ceratostomella*, herein described, which causes the blue stain. A study was made also of some yeasts that are apparently associated with the beetles.

Dendroctonus monticolae is one of the destructive insects of the western forests. It is found killing pines in the Sierras of California from the south to the north, and in the forests of Oregon, Washington, Idaho, Montana, and western Canada. The beetle attacks living trees and yearly with its associated fungus kills many acres of lodgepole pine (*Pinus contorta latifolia* Engelm.), possibly accounting for the relatively short life of these trees (*4*). It also kills sugar pine (*P. lambertiana* Dougl.), western white pine (*P. monticola* Dougl.), and ponderosa pine (*P. ponderosa* Dougl.).

Dendroctonus ponderosae also is regarded as one of the most aggressive and destructive of bark beetles. It is found in the mountain forests of western South Dakota, southeastern Montana, Wyoming, Colorado, Utah, and northern Arizona and New Mexico. Its favorite host is ponderosa pine, but it infests other pines, such as lodgepole and limber pine (*Pinus flexilis* James), when they occur with ponderosa (*1*). It was the cause of two historic epidemics recorded by the early settlers of the Rocky Mountain region, when acres of trees were killed in the Black Hills and Kaibab National Forests. Investigations by entomologists show that there have been a series of bark beetle epidemics extending through the years. The examination of a 400-year-old pine showed by the pitch pockets in its wood that it had survived seven series of unsuccessful beetle attacks. Hopkins (*9*) wrote:

> Very conclusive evidence has also been found that some of the great denuded areas in the Rocky Mountain region supposed to have been caused by forest fires were primarily caused by one or more species of *Dendroctonus*.

[1] Received for publication December 31, 1940.

[2] The investigations on which this paper is based were conducted in cooperation with the Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine, and the Forest Products Laboratory, Forest Service, U. S. Department of Agriculture.

[3] Italic numbers in parentheses refer to Literature Cited, p. 600.

MATERIALS AND METHODS

The specimens studied were collected by entomologists, who selected specimens of *Dendroctonus monticolae* within the region in the northern Rocky Mountains where this bark beetle is distributed, and of *D. ponderosae* within its Rocky Mountain region. They also furnished specimens collected in areas outside of their recognized territories and in territories where some of the beetles are atypical.[4]

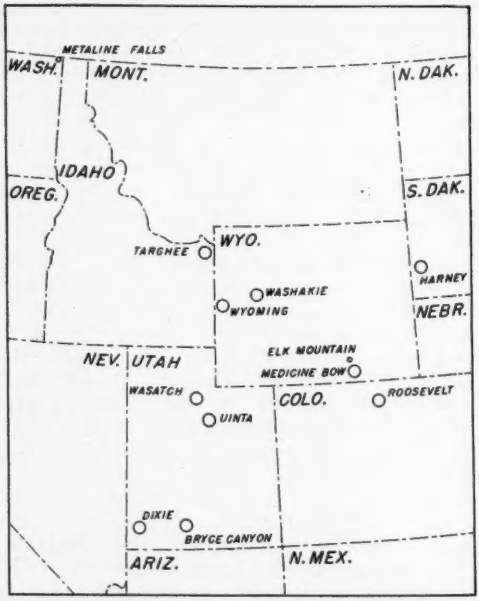

FIGURE 1.—Map of regions where bark beetles and blue-stained wood were collected.

Figure 1 shows the location of forests where collections were made of bark beetles and blue-stained wood used in the investigations here reported. As in previous studies (*15*, *16*), cultures were started by transferring to malt agar [5] insects, eggs, larvae, gallery walls, frass, or fragments of blue-stained bark, and wood. As soon as ascospores were produced they were planted, by the dilution method, on malt-agar plates from which single colonies were transferred to malt-agar slants for use in this study. The final selection of the isolate from its group depended on its vigor of growth and fructification. The isolates of *Ceratostomella* used in describing the fungus and for taking measurements were grown on malt agar and kept where the temperatures ranged from 12° to 17° C.

Table 1 gives the source and history of the isolates and dimensions of the perithecia when grown on malt agar.

[4] Entomologists record as atypical those specimens of a bark beetle infestation which do not show all the usual characteristics of a species.

[5] Formula for malt agar: Malt extract (Trommer's plain), 25 gm.; agar, 15 gm.: and water, 1,000 cc. The acidity is pH 5.5 to 5.6.

TABLE 1.—*Dimensions of perithecia of Ceratostomella montium isolated from Dendroctonus monticolae, D. ponderosae, or wood infested with them, and grown on malt agar*

Source of culture	Fruits measured	Height of base		Width of base		Length of neck	
		Usual range[1]	Mean	Usual range	Mean	Usual range	Mean
Dendroctonus monticolae, in region of usual distribution, on—	*Number*	μ	μ	μ	μ	μ	μ
Pinus monticola, Metaline Falls, Wash.	71	249–363	277	240–308	268	1,158–1,762	1,445
P. contorta latifolia, Targhee National Forest, Idaho	573	240–319	279	226–311	267	1,167–1,817	1,487
P. flexilis, Washakie National Forest, Wyo.	73	260–329	296	232–319	278	1,217–2,211	1,681
P. contorta latifolia, Wyoming National Forest, Wyo.	4	246–260	260	250–261	260	1,510–1,600	1,532
Total, range, or mean (14 cultures)	721	243–320	280	228–311	268	1,170–1,853	1,503
Dendroctonus monticolae, south of its recognized territory, on—							
Pinus contorta latifolia, Provo River area, Wasatch National Forest, Utah	359	275–356	314	258–340	299	1,190–2,001	1,581
P. contorta latifolia, Tabbie Mountain, Uinta National Forest, Utah	110	274–348	309	257–336	297	1,228–2,130	1,657
Total, range, or mean (4 cultures)	469	274–353	312	258–339	298	1,190–2,020	1,599
Dendroctonus ponderosae, in region of usual distribution, on—							
Pinus ponderosa, Dixie National Forest, Utah	187	246–328	286	231–317	275	1,004–1,451	1,251
P. ponderosa, Bryce Canyon National Park, Utah	191	245–336	288	229–321	277	1,010–1,737	1,416
P. ponderosa, Roosevelt National Forest, Colo.	75	239–300	272	231–308	267	923–1,215	1,044
P. ponderosa, Harney National Forest, S. Dak.	90	253–335	289	241–318	280	992–1,490	1,227
Total, range, or mean (11 cultures)	543	245–330	285	231–318	275	979–1,571	1,277
Dendroctonus ponderosae, territory where some of the beetles are atypical,[2] on—							
Pinus flexilis, Elk Mountain, Carbon County, Wyo.	264	256–333	293	240–325	281	1,168–1,857	1,521
P. contorta latifolia, Elk Mountain, Carbon County, Wyo.	379	243–322	282	230–309	269	1,202–2,048	1,621
P. ponderosa, Medicine Bow National Forest, Wyo.	289	262–324	293	252–311	281	1,439–2,204	1,820
Total, range, or mean (22 cultures)	932	250–328	288	240–317	276	1,241–2,083	1,654
Total, usual range, or mean for all strains	2,665	250–330	287	238–320	278	1,141–1,902	1,527

[1] Usual range discards the sixth of the measurements at each end of the extreme range, permitting an approximation of the frequency distribution.
[2] See footnote 4, p. 590.

THE FUNGUS IN ITS NATURAL HABITAT

Both *Dendroctonus monticolae* and *D. ponderosae* bore through the outer bark of the living tree to the region of the cambium and there extend their galleries. The first galleries in the inner bark and cambium are long vertical tunnels mined by the adults; from these extend smaller horizontal galleries made by larvae. These have the effect of girdling the tree.

The fungus begins its growth in the galleries and penetrates the inner bark and wood. Perithecia develop on the walls of the galleries. The bases of these fruits are generally buried in the bark, wood, or packed frass, and their necks extend into the galleries (fig. 2, *A*). They develop in both the beetle and larval galleries, where active beetles and larvae are smeared with the spores. The fungus grows in toward the heartwood, staining the sapwood.

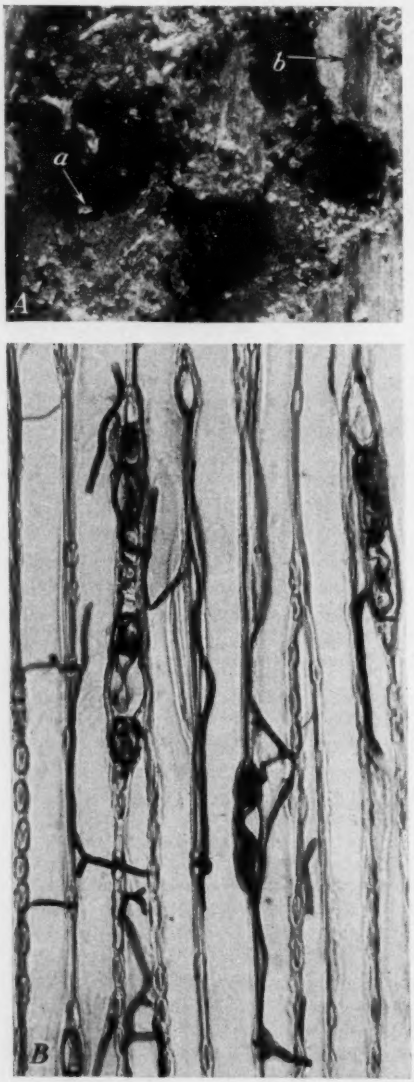

FIGURE 2.—*A*, Galleries of *Dendroctonus monticolae* in inner bark of *Pinus contorta latifolia*: *a*, Necks of perithecia extending into the galleries, with clumps of white ascospores on the tips of the necks; *b*, blue stain spreading from the galleries. × 15. *B*, Tangential section of *P. contorta latifolia* sapwood infected with *Ceratostomella montium* associated with *D. ponderosae*. Blue stain hyphae in rays and tracheids. × 400.

The color of the stain was not affected by the species of bark beetle or of the infested pine (*Pinus contorta latifolia*, *P. monticola*, *P. ponderosa*, or *P. flexilis*). Depending on the age of the infection and the moisture in the specimen, the colors ranged from Hathi gray (*14*) to storm gray, castor gray, dusky green gray, and blackish green gray. The color of the stain in the wood when received was usually castor gray. The rays become black.

As the fungus grows from the insect galleries it first invades the ray cells of the sapwood and later the tracheids, passing from cell to cell through the pits (fig. 2, *B*). Occasionally the hyphae penetrate the cell walls.

THE FUNGUS IN CULTURE

GROWTH

The young colonies are white. When the hyphae are about 6 days old their color begins to change to a warm sepia. Gradually the depressed mycelium turns black, while often light-gray aerial mycelium grows over part of the mycelial surface. On malt-agar slants the fungus often develops a thick white or cream-colored aerial mycelium at the top of the slant, which with age occasionally changes color to cartridge buff. As the culture ages, this aerial mycelium changes to wood brown in color or dries and becomes depressed. Some of this mycelium produces innumerable conidia on simple conidiophores; some of it is sterile. In the center of the slant the mycelium is olive brown,

then black. The necks of perithecia often extend through the blotches of dark-gray aerial mycelium. Mycelium has been found growing 1½ inches below the surface of the medium.

MYCELIUM

The hyaline hyphae in the young culture are septate, branch irregularly, and measure 1.3μ to 4μ in diameter. They form strands as they age, anastomose, and turn brown. Cell divisions become numerous, so that old hyphae sometimes consist of rows of short, slightly globular cells. The walls thicken, and sometimes a dark granular exudation appears. Old brown hyphae may be relatively thick, varying from 4μ to 8μ in diameter.

CONIDIA AND CONIDIOPHORES

The hyaline conidia, globular or ovoid, usually start developing 24 hours after the ascospore germinates. The globular conidia grow in

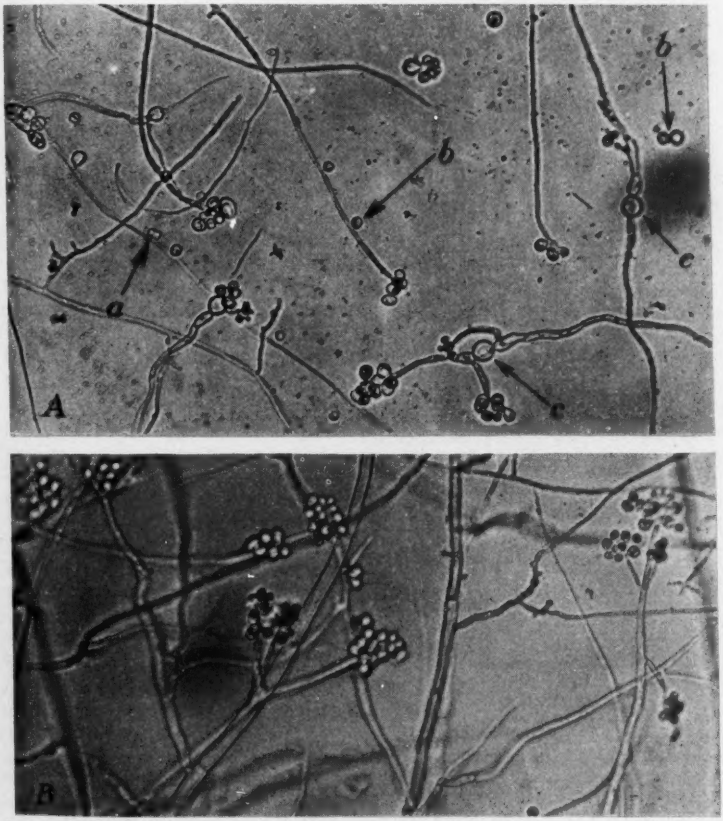

FIGURE 3.—Van Tieghem cell culture. *A*, Two days old, showing (*a*) ascospore, rectangular parallelepipedal with square ends; (*b*) globular ascospores; and (*c*) germinated ascospores with conidia developing in clusters on the hyphae. × 360. *B*, Four days old, showing simple branching of conidiophores and clusters of conidia. × 420.

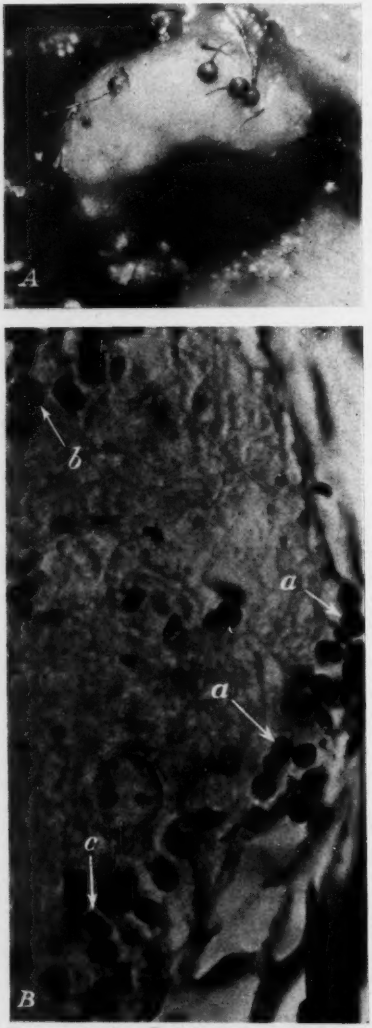

FIGURE 4.—*A*, Perithecia on and in the body of a larva of *Dendroctonus monticolae*. The live larva was taken from a gallery mined under the bark of a lodgepole pine and was dropped into a test tube containing malt agar. × 14. *B*, A section through the interior of the base of a young perithecium: *a*, Immature ascospores developing outside the ascus; *b*, somewhat older ascospores changing from globular to cylindrical; *c*, almost mature ascospore changing in shape to a rectangular parallelepiped with square ends. × 1,750.

small clusters on the tips of short conidiophores or on the ends of hyphae and are 4μ to 5μ in diameter. Ovoid conidia, 4μ to 5μ wide by 6.5μ to 8μ long, form on the germ tubes, directly on the hyphae, or on short conidiophores (fig. 3, *A* and *B*).

When 1 month old, Van Tieghem cell cultures had produced increased numbers of conidia in the clusters and along the sides of the hyphae but the conidiophores remained simple. No culture that was started from a single conidium produced perithecia. About 30 such cultures were made from one isolate.

PERITHECIA

The perithecia of this fungus are the largest of any *Ceratostomella* so far found associated with bark beetles.

The largest and most abundantly sporulating perithecia developed on the bodies of adults that were dropped into test tubes containing malt agar, where they were used as inocula. Live larvae were also good inocula and substrata (fig. 4, *A*). When the cultures were 6 months old it was difficult to dissect the perithecia from the insects because of the toughness of the mycelium in the body. In 9-month-old cultures the beetle and larvae bodies were partly digested. Usually after the cultures were 1 year old the bodies of beetles and larvae had been digested, and it was fairly easy to remove the whole perithecium from the black mycelium.

The shape of the perithecium is typical of *Ceratostomella*. The black or dark-brown globular base is usually submerged partly or wholly in the substratum on which it is growing and is commonly bare of basal hyphae except those that attach it to the substratum (fig. 4, *A*). However, perithecia are found occa-

sionally in culture with basal hyphae growing into the air, looking like bristles. These are short, straight, septate, and brown, and taper at the tip.

The long slender neck is black or dark brown, usually lighter colored at the tip. Rarely it divides into two or three necks. Three rows of closely packed ascospores can pass through the neck at once. The ostiole is without cilia. The dimensions of the perithecia are given in table 1. In general, the bases of the perithecia in the strains associated with *Dendroctonus monticolae* and *D. ponderosae* in the regions of their usual distribution and beyond did not differ significantly in size. The necks were slightly shorter in those strains associated with *D. ponderosae*, but this difference is not regarded as significant because the lengths of necks of *Ceratostomella* perithecia are usually more variable than the sizes of the bases.

ASCI

The immature asci seen were clavate or ellipsoid and contained eight immature ascospores. The ascus wall disappears before the ascospores are mature (fig. 4, *B*). The ascospores develop to a large extent outside the ascus. Their formation appears to keep pace with the growth of the perithecial neck, as was the case with other insect-blue stain fungi studied previously (*15*, *16*).

ASCOSPORES

The hyaline ascospores have the shape of rectangular parallelepipeds with square ends, and their angles are made prominent by flanges on the outer walls. They are covered with a thin coating of a mucilaginous substance. The ascospores resemble those of *Ceratostomella ips* Rumbold, another blue-staining fungus associated with insects (*16*, *fig. 5*).

Table 2 gives the dimensions of the ascospores of 20 isolates from different localities. The fungus cultures had been grown under similar laboratory conditions and were of about the same age when the perithecia were mounted for study. The length of the ascospores of all the isolates was practically the same; the width of the ascospores of the isolates from pines infested with *Dendroctonus ponderosae* in the region where this insect is usually distributed was about 4.6 percent smaller than those of the three other groups. Mathematical study showed that this difference in width is so small that it could be found between geographic strains of the same fungus even when associated with the same substratum and carrier. It is too small to use as a basis for distinguishing two species of fungi.

The ascospores did not germinate in sterile distilled water, but about 50 percent germinated within 24 hours in 3-percent malt solution or on malt agar. When ready to germinate the right square prism-shaped spore swells to more than twice its original size, becomes oval or round, and will produce 1 to 4 germ tubes (fig. 3, *A*, *b* and *c*).

In 2-day-old Van Tieghem cell cultures one finds ungerminated ascospores as well as some ascospores which have swollen and some which have germinated and produced hyphae and conidia. Figure 3, *A*, also illustrates the difference in size between germinated and ungerminated ascospores.

TABLE 2.—*Dimensions of ascospores of Ceratostomella montium associated with Dendroctonus monticolae and D. ponderosae*

Source of culture	Length of ascospore [1]		Width of ascospore	
	Usual range	Mean	Usual range	Mean
Dendroctonus monticolae:				
In region of usual distribution:	μ	μ	μ	μ
Metaline Falls, Wash.	4.4–5.1	4.6	2.4–3.0	2.8
Targhee National Forest, Idaho	4.4–5.1	4.6	2.4–3.0	2.7
Do.	4.4–4.7	4.6	2.4–3.0	2.8
Do.	4.1–5.4	4.7	2.4–3.0	2.8
Washakie National Forest, Wyo.	4.4–4.7	4.5	2.7–3.0	2.8
Do.	4.4–5.1	4.7	2.4–3.0	2.6
Wyoming National Forest (Greys River), Wyo.	4.4–5.1	4.7	2.4–3.0	2.6
Total of 140 measurements, usual range, mean	4.4–5.1	4.6	2.4–3.0	2.7
South of its recognized territory:				
Uinta National Forest, Utah	4.4–5.1	4.8	2.4–3.0	2.7
Do.	4.1–5.1	4.6	2.4–3.0	2.8
Wasatch National Forest, Utah	4.4–5.1	4.7	2.4–2.7	2.6
Do.	4.4–5.1	4.6	2.4–3.0	2.7
Total of 80 measurements, usual range, mean	4.4–5.1	4.7	2.4–3.0	2.7
Dendroctonus ponderosae:				
In region of usual distribution:				
Dixie National Forest, Utah, near Cedar City	4.4–5.1	4.8	2.4–2.7	2.6
Do.	4.4–4.7	4.6	2.4–3.0	2.7
Bryce Canyon National Park, Utah	4.1–5.1	4.6	2.4–2.7	2.6
Do.	4.4–5.1	4.7	2.0–2.7	2.5
Roosevelt National Forest, Colo.	4.4–5.4	4.8	2.4–2.7	2.6
Harney National Forest, S. Dak., near Custer	4.1–5.1	4.5	2.4–2.7	2.5
Do.	4.4–5.1	4.6	2.4–3.0	2.7
Total of 140 measurements, usual range, mean	4.1–5.1	4.7	2.4–3.0	2.6
Territory where some of the beetles are atypical: [2]				
Elk Mountain, Carbon County, Wyo.	4.4–5.1	4.6	2.4–3.0	2.8
Medicine Bow National Forest, Wyo.	4.4–5.1	4.6	2.4–3.0	2.7
Total of 40 measurements, usual range, mean	4.4–5.1	4.6	2.4–3.0	2.8
Total of 400 measurements, usual range, mean	4.4–5.1	4.6	2.4–3.0	2.7

[1] In every case these figures are based on the measurements of 20 ascospores.
[2] See footnote 4, p. 590.

TEMPERATURE RELATIONS

Ceratostomella montium is adversely affected by high temperatures and low humidities. Twelve of the first sets of *Dendroctonus monticolae*-infested pine specimens collected between 1930 and 1933 produced no fungi when cultured, while from other sets *C. montium* was occasionally isolated. In 1935, 1936, and 1937, when specimens and cultures were held in incubators at temperatures of 12° to 17° C., the cultures of 18 sets of *D. monticolae*-infested wood or of adult beetles produced *C. montium*. Also, cultures made from specimens of *D. ponderosae*-infested wood or from adult beetles developed *C. montium* when held at 12° to 17°. The cultures grew and fruited best when held in an incubator at a temperature of 16°. At this temperature the beetles and larvae used as inocula remained alive for 2 weeks. The fungus grew well, however, when held in incubators at temperatures between 12° and 22°. When the temperature was raised to 27° the mycelium began to deteriorate and yeasts grew vigorously. According to a Weather Bureau map defining the climatic provinces in

the central Rocky Mountains, the mean temperature where these specimens were collected is approximately 50° F.[6]

TECHNICAL DESCRIPTION

Ceratostomella montium n. sp.[7]

Young colonies with conidia white, changing to warm sepia and black, producing perithecia; young hyphae hyaline, 1.3μ to 4μ in diameter; old hyphae brown, septate, 4μ to 8μ in diameter; conidiophores single, hyaline, at first unbranched, later simple branched hyphae bearing conidia in clusters; conidia hyaline, clustered or solitary, appearing on hyphae and short conidiophores, globular, 4μ to 5μ, ovoid to clavate, 6.5μ to 8μ by 4μ to 5μ; perithecia black, globose, slightly hirsute; height of base, range 175μ to 448μ, usual range 250μ to 330μ, mean 287μ; width of base, range 162μ to 410μ, usual range 238μ to 320μ, mean 278μ; neck longitudinally striate; length of neck 552μ to 3,776μ, usual range 1,141μ to 1,902μ, mean 1,527μ; mean width of neck at tip, 21μ; the ostiole without cilia; asci ephemeral, clavate or ellipsoid; ascospores 8, one-celled, hyaline, rectangular parallelepipedal with square ends; length, range 3.7μ to 5.8μ, usual range 4.4μ to 5.1μ, mean 4.6μ; width, range 2.0μ to 3.4μ, usual range 2.4μ to 3.0μ, mean 2.7μ.

On sapwood of *Pinus monticola*, *P. contorta latifolia*, and *P. flexilis* infested with *Dendroctonus monticolae*, and of *P. ponderosa*, *P. flexilis*, and *P. contorta latifolia* infested with *D. ponderosae*, in forests in the central Rocky Mountains, United States of America.

DIAGNOSIS

Ceratostomella montium sp. nov.

Coloniis juvenilibus conidiferis albis tum brunneis-nigrescentibus cum perithe ciis; hyphis hyalinis 1.3μ–4μ diam., deinde brunnescentibus 4μ–8μ diam.; conidiophoris hyalinis, simplicibus ramosis; conidiis hyalinis, in hyphis primo apparentibus, globosis 4μ–5μ, ovoideis vel clavatis 6.5μ–8μ × 4μ–5μ, solitariis, deinde in massulas congregatis; peritheciis nigris, globosis, leniter hirsutis, 175μ–448μ altis, 162μ–410μ latis; rostellis 552μ–3,776μ longis, ostiolo carente filamentis; ascis evanidis, clavatis, octosporis, ascosporis hyalinis, formis rectangular parallelepiped, quadratis extremis, 3.7μ–5.8μ × 2μ–3.4μ.

In ligno sapido *Pinus monticola*, *P. contorta latifolia*, *P. flexilis Dendroctono monticolae* infestato, et in ligno sapido *P. ponderosa*, *P. flexilis*, *P. contorta latifolia Dendroctono ponderosae* infestato.

Silvis in mediis Rocky Mountains, United States, America.

YEASTS ASSOCIATED WITH CERATOSTOMELLA MONTIUM

From all of the inocula yeasts [8] developed first, and later *Ceratostomella montium* appeared, growing out from the yeast colonies (fig. 5, *A*). They were of the same two types of yeast associated with the bark beetles that previously had been studied for fungus associations (*7*, *15*, *16*). In the laboratory the yeasts endured and grew at higher temperatures than *C. montium*.

Most of the yeasts belonged to species of *Monilia*. The term *Monilia* here means, according to Guilliermond (*5*), Henrici (*6*), and Stovall and Bubolz (*18*), a non-spore-forming, or anascosporous,

[6] In 1903 Von Schrenk (*20*) described a blue stain fungus, *Ceratostomella pilifera* (Fr.) Wint. which he isolated from ponderosa pines infested with *Dendroctonus ponderosae* collected in the Black Hills Forest Reserve, S. Dak. (now the Harney National Forest), but he was unsuccessful in isolating a blue-staining fungus from the beetles. The high temperatures in the laboratory at the Missouri Botanical Garden, St. Louis, Mo., where the cultures were made, may have suppressed the growth of *C. montium* and encouraged that of *C. pilifera*. Scheffer and Lindgren (*17*) have shown that geographical strains of *C. pilifera* differ measurably in their reaction to temperature. The maximum rate of growth of several strains has been found to occur at 28° to 29° C., while growth is inhibited at 35°.

[7] Since this paper was prepared, the name *Ceratostomella montium* Rumbold has been used by Vinje (*19*), to whom the writer supplied cultures and who described the development of the perithecia.

[8] These yeasts were cultured and identified by Eugene C. Holst, formerly student assistant, Division of Forest Pathology, now assistant bacteriologist, Bureau of Entomology and Plant Quarantine.

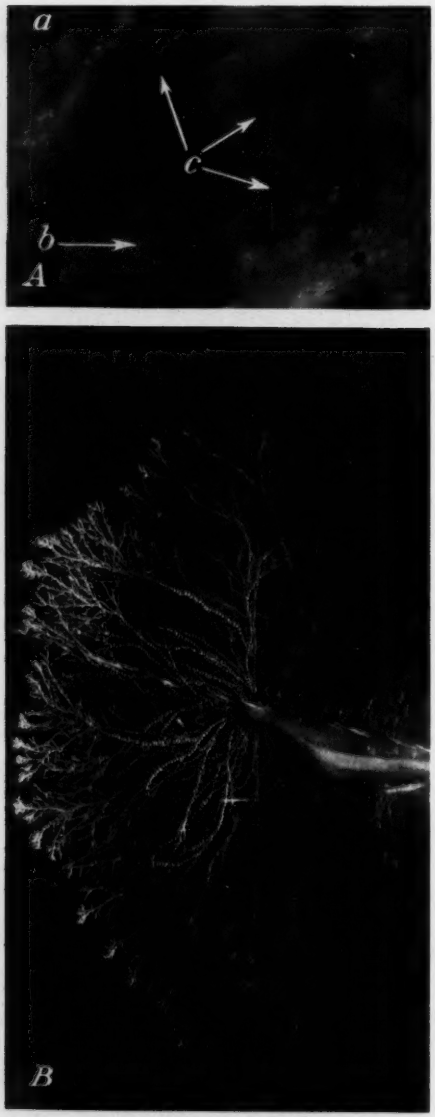

FIGURE 5.—*A*, Culture on malt agar of *Ceratostomella montium* and anascosporous yeasts from an adult *Dendroctonus ponderosae*: *a*, Black mycelium; *b*, white glistening yeast; *c*, black perithecia growing on surface of yeast. About × 10. *B*, A 9-day-old culture of anascosporous yeast isolated from an adult *D. ponderosae*. × 12.

budding yeast, which may also exist in a mycelial form (fig. 5, *B*) with the possibility of conidia being borne on the hyphae.

These anascosporous yeasts are mostly *Mycocandida* and *Mycotoruloides* types, defined by Langeron and Talice (*10*) and Lodder (*12*). One of the types isolated from *Dendroctonus monticolae* collected in the Metaline Falls, Wash., region was found to resemble a type isolated from the insect *D. piceaperda* Hopk. collected in Canada.[9]

In addition to the usual anascosporous mycelium-forming yeasts, *Zygosaccharomyces pini* Holst (*?*) was isolated from seven *Dendroctonus ponderosae* beetles collected in Harney National Forest, S. Dak.

The different species of yeasts grew on sterilized pine on which the blue stain fungus did not grow. They have a stimulating effect on the staining fungus, causing it to grow more vigorously on agar and to fruit more quickly than the pure cultures of *Ceratostomella montium*. They are frequently found growing up the outside of the necks of the perithecia, so that when ascospores are ejected from the ostioles, yeast is mixed with them.

DISCUSSION

Ceratostomella montium is probably too sensitive to environmental influences

[9] HOLST, E. C. YEASTS ASSOCIATED WITH SEVERAL SPECIES OF BEETLES. Prog. Rpt., Div. Forest Path., U. S. Bur. Plant Indus., July 1, 1934. [Unpublished manuscript.]

to be able to spread far from the mountain forests. In this it differs from *C. ips*, which can grow in a wide range of temperatures (10° to 35.5° C.) and can infect freshly sawed boards in southern lumberyards. While the mycelium of *C. montium* can permeate malt agar for 1½ inches below its surface, its perithecia develop only on the surface of the medium. Perithecia of *C. ips* are frequently found beneath the agar surface. Though the perithecia and ascospores of *C. montium* are larger than those of *C. ips*, whose height of base of perithecium averages 206μ, width 198μ, and length of neck 1,273μ, this fungus looks like *C. ips*, in many respects, such as the lack of cilia at the ostiole, the shape of the ascospore, rectangular parallelepiped with square ends, appearance of germinating ascospores, and formation and shape of conidia. This fungus does not, however, produce either the well-developed conidiophores or the fascicles of conidiophores which develop in cultures of *C. piceaperda* and *C. ips*. Its conidiophores are simple, and in this phase of growth it resembles *C. pseudotsugae* Rumbold (*15*.)

An association of the same blue stain fungus with more than one species of bark beetle is not unusual. *Ceratostomella pini* Münch is associated with *Dendroctonus frontalis* Zimm. on the Atlantic coast and with *D. brevicomis* Lec. on the Pacific coast. *C. ips* is associated with different species of *Ips* on the Atlantic and Pacific coasts and in the Central States (*11*). Whether *C. montium* is associated with *D. monticolae* found in the forests in the Sierras on the Pacific coast or in Canada or with *D. ponderosae* in the Kaibab National Forest or in Mexico is not known.

The two types of yeast, *Zygosaccharomyces pini* and the anascosporous yeasts, that were found regularly associated with *Dendroctonus monticolae* and *D. ponderosae*, are those found with other species of bark beetles. They appear to be widespread in the forests in this country. They are regarded as of secondary importance in causing the death of insect-infested pines. Experiments of Nelson and Beal (*13*) showed that blue stain fungi inoculated into pine trees killed them without the aid of bark beetles or yeast. Similar experiments by Bramble and Holst (*2*) gave the same result. They found that when inoculated into pines the yeast *Zygosaccharomyces pini* showed no evidence of its pathogenicity. Holst (*8*) found that the life of the bark beetle *D. frontalis* was not dependent on yeast, as he raised sterile larvae and an adult from surface-sterilized eggs.

It was noticed that when there was an epidemic of *Dendroctonus monticolae* and *D. ponderosae* in the forest the specimens sent in, whether insects or the bark or sapwood of infested pines, developed *Ceratostomella montium* and yeast in almost pure form. When the beetle infestation in the forest was light, however, the *C. montium* cultures isolated grew slowly and other fungi also were isolated. The close association of beetle and fungus could be seen in the original test-tube cultures when adults or larvae were the inocula. They developed vigorous cultures of *C. montium*, with large perithecia growing on the bodies of insects and larvae.

SUMMARY

The bark beetle *Dendroctonus monticolae* infests mountain pines in the central Rocky Mountain forests, extending from the Canadian

boundary south to the Targhee National Forest in eastern Idaho to the Wyoming and Washakie National Forests in southwestern Wyoming and the Wasatch and Uinta National Forests in northeastern Utah. It carries with it in its attacks on the pines a blue stain fungus, *Ceratostomella montium*, a *Ceratostomella* not previously described. This fungus is disseminated also by the bark beetle *D. ponderosae* when it infests pines in mountain forests extending from Dixie National Forest in southwestern Utah, Bryce Canyon National Park in south-central Utah, and in forests extending northeast to the Roosevelt National Forest in northeastern Colorado, Medicine Bow National Forest in southeastern Wyoming, and Harney National Forest in southwestern South Dakota. It is not known whether this fungus is associated with the beetles in other parts of their range.

It is doubtful whether *Ceratostomella montium* will spread from the mountain forests, for in artificial culture this fungus does not tolerate high temperatures. It has been found to grow best at a temperature of 16° C., and grew well between 12° and 22°.

Ceratostomella montium grows beneath the bark of beetle-infested pines. At the time the insects are emerging, the long necks of the large perithecia grow into the galleries, where they appear like thin black wires. The fungus-infected sapwood usually is gray, shading to blackish green gray.

The beetles carry with them, besides the blue stain fungus, two types of yeast—an anascosporous mycelium-forming group and *Zygosaccharomyces pini*.

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EFFECT OF TEMPERATURE DURING IRRADIATION ON THE X-RAY SENSITIVITY OF MAIZE SEED[1]

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INTRODUCTION

Certain organisms in the resting phase respond to heavy X-ray dosages by dying soon after an apparently normal early growth. In the case of air-dry maize seeds this phenomenon, which has been called delayed death (*1*),[2] is characterized by death of the seedling following normal germination and development to a stage where the first leaf is partially exserted from the coleoptile. This behavior has been noted for all the common cereals and numerous dicotyledonous species. Comparable behavior has been observed in the fungus *Ustilago hordei*.[3] Sugiura (*12*) has reported a similar response for wheat seedlings, and Enzmann and Haskins (*4*) have noted delayed death for many garden seeds and also for the vinegar fly, *Drosophila*.

Tests for the possible destruction of growth substances indicate that auxin production is not directly interfered with by the irradiation used, although there is evidence that the transportation mechanism is restricted.[4] It is believed, therefore, that delayed death cannot be explained as a secondary reaction brought about by the lack of growth substances. A break-down in transportation, however, points to a disruption of cellular dynamics, of which the failure to transport growth substance is one manifestation.

A possible explanation of the partial growth but early death of seedlings from X-rayed seeds can be based on the profound derangement of the chromatin produced by X-rays. It has been shown by Stadler (*10*) and others that chromosomes are broken as the result of X-raying seeds and that these breaks lead to translocations, inversions, deletions, and fragmentations.

Whiting (*13*), experimenting with irradiated unfertilized eggs of *Habrobracon*, concluded that the death of eggs is correlated with their chromatin condition. She found that eggs in the first meiotic metaphase failed to hatch, that those in the latest prophase (diakinesis) hatched but failed to mature, and that those newly differentiated (preleptotene) had a mortality of 69.6 percent and a large percentage died as larvae. The younger oocytes with nurse and follicle cells (early prophase) are the most resistant with the exception of those in the late prophase with diffuse chromatin, which have the lowest mortality of all.

Stone (*11*) studied the effect of X-rays on the mitotic divisions in the root tips of *Crocus olivieri* and concluded as follows:

> It appears likely that the sequence of events is as follows. X-ray treatment causes a relatively spontaneous physiological reaction, which is sufficient to suppress

[1] Received for publication January 24, 1941.
[2] Italic numbers in parentheses refer to Literature Cited, p. 618.
[3] RODENHISER, H. A., and MAXWELL, LOUIS R. [Unpublished manuscript.]
[4] DU BUY, H. G. [Unpublished manuscript.]

further division in resting nuclei but not able to stop the process of division already begun. This is in agreement with Strangeways and Hopwood,[5] but it is possible to go further and suggest that this change increases slowly in intensity, and when the intensity is at its highest acts in a similar way upon the resting nuclei, derived from divisions allowed to proceed normally after treatment. Activity is suspended for a certain period (depending upon the dose applied), and then the effect wears off and mitoses begin to appear. Many are abnormal, and it is clearly possible that these abnormalities may have been initiated during the post-mitotic as well as during the pre-mitotic resting stages following treatment.

Collins and Maxwell (*1*) report that in root-tip material of delayed-killed maize seedlings Longley found all mitoses abnormal in some degree, the abnormalities ranging from divisions with lagging chromosomes to divisions in which the chromatin was an undifferentiated amorphous mass. Such nuclear disorganizations produce unequal distribution of chromatin when cells divide. Thus, although a few cell generations are possible with unequally divided chromosomes eventually the loss of balance between nuclear elements prevents further division and growth ceases. This idea finds support in the fact that under normal conditions the cells of the lower leaves of maize have a life limited at most to a few weeks. In the absence of continuing cell generations the first leaves of uninjured seedlings presumably die simply because the cells are short-lived. When normal maize seedlings have their growing point above the first true leaf removed, this leaf may survive 3 weeks—a period not much longer than the time seedlings from X-rayed seeds may live.

Although delayed death is a consistent result obtained with air-dry maize seeds for dosages greater than 60 kr.,[6] this is not the case when dosages approach the delayed-death threshold. In very exceptional cases 100 percent delayed killing has been obtained with a dosage as low as 20 kr., while in other instances a high percentage survived at 35 kr. In all these experiments the X-ray dosage was under rigid control, having a maximum variation of 5 percent. The erratic results in seedling survival at the threshold dose undoubtedly may be ascribed to important uncontrolled factors operative after planting. Some progress has been made in determining the factors of environment that affect survival, but the results are not yet consistent enough to justify conclusions.

More certain results have been obtained with temperature treatments given the seeds during the time of irradiation. It has been shown (*7*) that when seeds are held at liquid-air temperature during irradiation their sensitivity to X-rays is reduced, as determined by the extent of seedling growth in the delayed-killed stage. Apparently those factors responsible for delayed death are diminished by exposure to liquid-air temperature during irradiation.

The present paper reports further experiments on the effect of the temperature of air-dry seeds during the time of irradiation on the growth of maize (*Zea mays* L.) seedlings. The temperatures investigated ranged from −187° to 66° C.; X-ray dosages ranged from 35 to 45 kr.

EXPERIMENTAL METHODS

The X-ray set-up used for the irradiation has been described previously (*7*). The seeds were placed in a metal boat that floated on

[5] Citation to literature in Stone (*11*).
[6] Abbreviation of kiloroentgen; 1 kr. equals 1,000 roentgens.

liquid air for the −187° C. temperature, in a thick mixture of carbon dioxide snow and alcohol for the −66° temperature, in crushed ice and water for the 0° treatment, in water at room temperature for the room-temperature point, and in hot water for the 50° temperature. For the 66° temperature, an oven with temperature controls was used. Seed temperatures were determined by embedding a thermocouple in one of the seeds. Temperatures given are accurate within ±5° for the liquid air flask arrangement and ±2° for the oven. When the oven was used the seeds were lying on a sheet of paper; therefore the dosages given under these conditions were increased by about 5 percent to subject the seeds to the same dosage as that given in the liquid-air flask arrangement. Under the conditions of irradiation, i. e., with 45-kr. constant potential, tungsten anode, and no appreciable filtration, the amount of back-scattering was measured and was found to be about 8 percent. Dosage values are measured by an open air ionization chamber and are accurate within 5 percent. Exposure time ranged from 3½ to 5 hours.

The seed used throughout was Funk Yellow Dent drawn from the large, thoroughly mixed sample used in previously reported experiments. The lot from which the treated samples were drawn was stored at 1° to 2° C. In all the exepriments reported here, except 1, the seedlings were grown in a greenhouse in metal or rubber flats. These flats accommodated 144 seedlings in 12 rows of 12. They were 1 foot square and 4½ inches deep. After the seed was sown, the flats were mounted on compound clinostats, where both the table and the individual flats rotated independently. Tests were made in randomized blocks, each flat containing 2 or more complete series. Where the experiment was of such a size that more than 1 clinostat was required, the choice of flats between clinostats was also random.

With the exception of the last four experiments described below the seedlings were grown in carefully mixed soil covered with a half-inch layer of water-washed sand. Usually the variance between flats exceeded that which might be attributed to chance in spite of meticulous care in distributing the sterilized soil among the flats, in compressing it before planting, and in watering before and during the course of the experiments. Accordingly various tests were conducted with sand and soil, with and without culture solutions. The least variance between flats was obtained with sand saturated with the culture solution given by Eaton (*3*). Consequently this system has been used for the last four experiments reported. The seeds were planted point down, at a depth of one-half inch in soil or in water-washed sterilized sand brought to a moisture content of about 25 percent. The flats were covered with impervious black paper until the seeds germinated. Then the paper was removed and the flats were brought up to saturation each morning. In all cases the seedlings were measured at least twice and in some instances five times, at intervals of 2 days. Failure to elongate between any two measurements indicated death. Most of the experiments were continued until all question of survival or demise was settled.

The variance was analyzed for each series of measurements for each experiment, and the errors given below are from generalized standard deviations for each experiment. As a rule the variance of blocks within flats and between clinostats never exceeded that expected

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by chance, and in the last four experiments this was true of the variance between flats.

RESULTS

The results are presented in chronological order. This is done to illustrate the reproducibility of the effects observed and also because the experiments lead from one to another.

EXPERIMENT 1, THREE-POINT LARGE-TEMPERATURE-VARIATION TESTS, APRIL 1939

Three lots of 288 seeds each were given a dosage of 35 kr. These were held at −187° C., room temperature, and 61°, respectively, during the time of treatment. The seeds were planted on April 10, 1939, and the experiment was terminated on April 20, since by that date the seedlings from seeds X-rayed at room temperature were dying. By April 26 all the seedlings of the room-temperature series were dead. No final count of survivors was made, because some of the flats were broken down for photographing. A summary of the results obtained is given in table 1.

TABLE 1.—*Effect of seed temperature during X-raying (35 kr.) on size of seedlings, April 1939*

Seed temperature (° C.) at exposure to X-rays	Total seedlings [1]		Mean height of seedlings	
	Apr. 17	Apr. 20	Apr. 17	Apr. 20
	Number	*Number*	*Millimeters*	*Millimeters*
−187	254	253	19.30±0.74	27.41±1.53
21 (room)	279	279	8.51± .71	6.44±1.46
61	285	284	35.66± .70	81.82±1.45

[1] 288 seeds in each treatment.

It is noticed that, in agreement with results previously reported (7), the sensitivity of seeds held at −187° C. was definitely less than the sensitivity of seeds kept at room temperature. The seeds maintained at 61°, however, produced plants having the greatest height. Figure 1 shows a photograph of three plants typical of the three temperature groups. Chlorophyll deficiencies may be noted in the surviving plants.

To determine the effect of temperature alone, a duplicate set of 288 seeds was held at each of these temperatures for the same length of time as the X-rayed series (3½ hours) and in the same containers and positions. These non-X-rayed seeds were planted April 13 in the same manner as those of the X-rayed series and were measured April 21. Each lot of seedlings from this non-X-rayed material was measured only once, and the results are shown in table 2.

TABLE 2.—*Effect of seed temperature without X-rays on size of seedlings, April 1939*

Seed temperature (° C.) at exposure	Total seedlings [1]	Mean height of seedlings
	Number	*Millimeters*
−187	235	103.08±1.59
21 (room)	280	140.96±1.46
61	281	139.43±1.46

[1] 288 seeds in each treatment.

It is seen that heating the seeds to 61° C. for 3½ hours had no effect on the size of the seedlings but that exposure to low temperature had a pronounced detrimental effect on both germination and size. From

FIGURE 1.—Typical plants selected from experiment 1 to illustrate temperature effects. Treatment temperatures during irradiation (35 kr.) were as follows: *A*, 21° C.; *B*, −187°; *C*, 61°.

table 1, it is evident that germination was low also for seeds X-rayed at −187°, but the plants which did appear grew definitely taller than those from seeds X-rayed at room temperature.

EXPERIMENT 2, THREE-POINT SMALL-TEMPERATURE-VARIATION TESTS, MAY 1939

In experiment 2, 2 X-ray dosages (30 and 35 kr.), 3 temperatures (20°, 50°, and 60° C.), and 6 lots of 48 seeds each were used. The seeds were planted on May 17, 1939, and the seedlings were measured on May 22, 24, 26, and 29. A summary of the results is given in table 3. By May 26 delayed death from some treatments was evident, so the mean heights are given in table 3 for the measurements made on May 24. The number of surviving seedlings was determined May 29.

TABLE 3.—*Effect of seed temperature during X-raying on size of seedlings and number of survivors, May 1939*

X-ray dosage (kiloroentgens)	Seed temperature at exposure	Total seedlings [1]	Mean height of seedlings	Seedlings surviving
	°C.	*Number*	*Millimeters*	*Number*
30	20	46	8.67±0.93	10
	50	42	51.89± .97	42
	60	45	47.56± .94	45
35	20	47	8.15± .92	1
	50	47	37.40± .92	43
	60	48	34.06± .91	46

[1] 48 seeds in each treatment.

It is obvious that treatments at temperatures of 50° and 60° C. resulted in increased height and number of survivors as compared with room temperature and that the difference in X-ray dosage of 5 kr. produced a significant difference in seedling size at the two higher temperatures. The interaction of dosage with temperature was not significant, a result to be expected since the two X-ray dosages were not greatly different. Although in both X-ray treatments the seeds held at 50° gave larger seedlings than those held at 60°, the differences within X-ray dosages are not significant. The possibility is suggested in this experiment that the most effective temperature for resistance to the effects of X-rays is between 50° and 60°.

EXPERIMENT 3, MOISTURE-SENSITIVITY TESTS, JUNE 1939

Since raising the temperature of seeds will reduce their moisture content, the next experiment in this series was designed to determine the effects of X-rays on seeds of low moisture content. For this purpose a sample of 288 seeds was dried in a vacuum oven at 70° C. until the moisture content had been reduced from 8 to 2 percent. One hundred and forty-four of these seeds were given an X-ray dose of 35 kr. at room temperature. The remaining 144 seeds were used as controls. There were also included in this experiment 144 stock seeds of 8-percent moisture subjected to 35 kr. of X-radiation at room temperature and an equal number not X-rayed. These four lots of seeds were planted on June 15, and the seedlings were measured on June 20, 21, 22, 23, and 26. The statistical treatment was confined to the measurement on June 22, at which date germination was complete and delayed death had not become apparent. The results are shown in table 4.

TABLE 4.—*Number and mean height of seedlings from oven-dried (2-percent moisture) and stock (8-percent moisture) seeds X-rayed and not X-rayed, June 1939*

Condition of seed	Treatment	Total seedlings [1]	Mean height of seedlings	Seedlings surviving
		Number	*Millimeters*	*Number*
Oven-dried (2-percent moisture)	Not X-rayed	123	126. 33±1. 06	123
	X-rayed (35 kr.)	90	11. 41±1. 22	0
Stock (8-percent moisture)	Not X-rayed	123	125. 49±1. 06	123
	X-rayed (35 kr.)	128	49. 87±1. 03	92

[1] Each lot of seeds treated totaled 144.

It will be noted that a reduction in the moisture content had no effect on seedling size when the seeds were not X-rayed, but when they were treated the deleterious effect of the drying was pronounced both with respect to survival and mean height of surviving plants. Insofar as this experiment can be considered typical, the beneficial effect of high temperature during irradiation cannot be ascribed to a reduction in moisture content.

EXPERIMENT 4, TWO-POINT SMALL-TEMPERATURE-VARIATION TESTS, AUGUST AND SEPTEMBER 1939

In experiment 4 an X-ray dosage of 45 kr. and seed temperatures of 31° C. (room temperature) and 51° were used. The choice of 51° instead of 60° was made because in experiment 2 (table 3) the seeds treated at 60° showed the least sensitivity to X-radiation. The seeds were heated in the oven mentioned above. Two hundred seeds were X-rayed at each temperature. Of these, 144 from each lot were grown in the greenhouse and 48 in an air-conditioned room maintained at approximately 25°, with continuous illumination at 300 foot-candles (Mazda). The plantings were made on August 26, and measurements were made August 31 and September 2, 5, and 7. At the second measurement, germination was complete and no seedlings had died. The results are shown in table 5.

TABLE 5.—*Effect of X-ray treatment (45 kr.) of seeds [1] on mean height and number of surviving seedlings, August and September 1939*

Seed temperature (° C.) at exposure to X-rays	Location of plants	Total seedlings	Mean height of seedlings	Seedlings surviving [2]
		Number	*Millimeters*	*Number*
31	Greenhouse	142	26. 13±0. 93	107
51	Greenhouse	141	29. 30± . 93	78
31	Air-conditioned room	47	25. 43±1. 62	
51	Air-conditioned room	47	31. 38±1. 62	

[1] 200 seeds were X-rayed at each temperature; of these, 144 seeds from each lot were grown in the greenhouse and 48 in an air-conditioned room at approximately 25° C., with continuous illumination at 300 foot-candles.

[2] The seedlings in the air-conditioned room could not be classified on a survival basis as could those in the greenhouse.

The differences in height of plants between the two treatment temperatures would be expected as the result of chance about once in 20 trials and therefore can hardly be considered significant. However, since both trials showed the same behavior and to about the same degree, somewhat more confidence may be placed in the results. At the fourth measurement, death had overtaken a large number of the

seedlings and, since the survivors were evidently capable of completing growth, the experiment was terminated.

For plants grown in the greenhouse, the χ^2 of the distribution of living and dead plants from the two seed treatments on the assumption that the two populations are random samples from the same lot, is 15.04; from this it is concluded that the seed temperature during irradiation was an important factor in determining whether the seedlings were to live or die. This confirms the beneficial effects derived from using high temperature during irradiation.

EXPERIMENT 5, SIX-POINT LARGE-TEMPERATURE-VARIATION TESTS, DECEMBER 1939

In experiment 5 an X-ray exposure of 45 kr. and temperatures during radiation of −187°, −66°, 0°, 27°, and 56° C., were used and 1 lot was X-rayed at 27° and was then heated at 60° for the same length of time as the others, namely, 4½ hours. Two hundred seeds were treated at each temperature, and 192 of each of these were planted in 8 flats on December 4. The results are shown in table 6.

TABLE 6.—*Effect of seed temperature during X-raying (45 kr.) on number of survivors and on height of seedlings grown in the greenhouse, December 1939* [1]

Seed temperature (° C.) at exposure to X-rays	Total seedlings	Mean height of seedlings [2]	Seedlings surviving
	Number	*Millimeters*	*Number*
−187	187	24.00	27
−66	184	18.09	0
0	187	14.96	0
27	190	14.53	0
60 after 27	187	15.90	0
56	189	19.46	3

[1] 200 seeds were treated at each temperature; 192 seeds from each lot were planted in 8 flats December 4, 1939.

[2] Standard error equals ±0.47.

The possible differences between these treatments are given in table 7.

TABLE 7.—*Differences in mean height between the seedlings from various seed temperatures* [1]

[Based on data in table 6]

Seed temperature (° C.) at exposure to X-ray	Differences between seedlings from indicated seed temperatures (° C.)				
	−187	−66	0	27	60 after 27
	Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*
−66	5.91				
0	9.04	3.13			
27	9.47	3.56	0.43		
60 after 27	8.10	2.19	−.94	−1.37	
56	4.54	−1.37	−4.50	−4.93	−3.56

[1] Minus sign indicates that the seedlings from the treatment shown at left were larger than those from the treatment designated by the column head.

As the standard error of a difference is ±0.67, it follows that differences to be significant must exceed 1.34 mm. Of the 15 differences, only 2 are below the level of significance, namely, the differences between seedlings from the seed temperatures 0° and 27° C., and

between seedlings from seeds X-rayed at 0° and from seeds given a heat treatment of 60° after being X-rayed at 27°. There are 2 other differences just over the border line of significance, namely, those between seedlings from seeds X-rayed at 27° and from seeds given a 60° heat treatment after being X-rayed at 27° and between seedlings from seed temperatures 56° and −66°.

From this experiment it must be concluded that the most sensitive seed temperature for the action of X-rays falls somewhere within the range of 0° to 27° C. In agreement with the results of previous experiments it was found that temperatures either above or below this point were effective in affording a degree of protection from the action of X-rays.

When the seedling flats of this experiment were broken down, it was observed that the length of the primary root varied with the seed treatment. Plants from two of the flats were lost before this observation could be put to the test, but the primary roots of the plants from the remaining six flats were measured, and the results are shown in table 8.

TABLE 8.—*Effect of seed temperature during X-raying (45 kr.) on length of primary roots*

Seed temperature (° C.) at exposure to X-rays	Total seedlings	Mean root length [1]	Seed temperature (° C.) at exposure to X-rays	Total seedlings	Mean root length [1]
	Number	*Millimeters*		*Number*	*Millimeters*
−187	137	51.91	27	140	37.28
−66	136	41.37	60 after 27	139	39.53
0	139	37.44	56	140	43.60

[1] Standard error equals ±1.05.

In general, these root data support the measurements of seedling height although the errors are greater, partly owing to the reduction in number. Possible differences are given in table 9.

TABLE 9.—*Differences in length of primary root between seedlings from various seed temperatures*

[Based on data in table 8]

Seed temperature (° C.) at exposure to X-rays	Differences [1] between seedlings from indicated seed temperatures (° C.)				
	−187	−66	0	27	56
	Millimeters	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*
−66	10.54				
0	14.47	3.93			
27	14.63	4.09	0.16		
56	8.31	−2.23	−6.16	−6.32	
60 after 27	12.38	1.84	−2.09	−2.25	4.07

[1] Minus sign indicates that the roots of seedlings from the treatment shown at left were longer than those from the treatment designated in the column head.

The standard error of a difference is ±1.48, so that to be considered significant differences must exceed 2.96. Of the 15 differences, 5 fall below the level of significance.

EXPERIMENT 6, SIX-POINT LARGE-TEMPERATURE-VARIATION TESTS, JANUARY 1940

This experiment was essentially a repetition of the one just discussed. One hundred and ninety-two seeds at each temperature were planted in 8 flats January 15, and measurements were made on January 22, 24, and 26. During this period the sky was overcast, permitting a fair control of greenhouse temperatures but also resulting in a greatly reduced illumination which was accentuated by snow that covered the roof for 2 days. At the time of the first measurement there was a significant lag in germination of the seeds treated at 50° C. and a depressed growth at this temperature. By the second measurement, 2 days later, the germination had increased though it was still below average. At the same time the plants increased in size, growing more rapidly than those from any of the other treatments. This difference persisted to the close of the experiment and remains unexplained. Since the treatments were randomized in 8 flats on 2 clinostats and the data were consistent, the relatively poor and greatly delayed germination of the seeds treated at 50° C. cannot be attributed to the growing conditions. It is equally certain that there was no aberration in handling during exposure to X-rays. For the dosages given, only 100 seeds can be X-rayed at a time, so that each of these lots of 192 seeds was made up of two separate exposures. These lots were kept separate in planting, and the analysis of variance shows that the paired exposures were as nearly alike as could be expected from random sampling.

Delay in germination had the effect of placing these 50° C. seedlings in an environment somewhat different from those of the other treatments. But since the greenhouse temperatures were fairly uniform, the chief difference was in the amount of light the plants received.

By the third measurement, on January 26, delayed death was apparent in many of the treatments. The data are given in table 10; errors were calculated for only the second measurement.

TABLE 10.—*Effect of seed temperature during X-raying on size of seedlings and number of survivors, January 1940*

Treatment		Total seedlings			Mean height of seedlings			Seedlings surviving	
Seed temperature (° C.) at exposure to X-rays	X-ray dosage	Jan. 22	Jan. 24	Jan. 26 [1]	Jan. 22	Jan. 24 [2]	Jan. 26	Jan. 29	Jan. 31
	Kr.	*No.*	*No.*	*No.*	*Mm.*	*Mm.*	*Mm.*	*No.*	*No.*
−187	35	192	192	191	12.95	16.89	17.30	45	18
−62	35	186	186	186	12.86	16.51	16.72	25	15
0	35	187	187	187	12.01	15.39	15.53	21	5
27	35	185	185	185	12.29	16.13	16.36	15	5
50	35	138	173	177	11.15	20.29	24.88	146	94
66	36.7	185	188	188	14.45	22.30	23.93	27	12

[1] One plant was broken in measuring.
[2] Standard error equals ±0.55.

EXPERIMENT 7, SIX-POINT LARGE-TEMPERATURE-VARIATION TESTS, MARCH 1940

In experiment 7 the temperatures duplicated those of experiment 6, but the X-ray dosage was reduced to 32.7 kr. except for the seeds treated at 66° C., in which case the dosage was 34.3 kr. This difference was applied to counteract the effect of back-scattering from the container used at the lower temperatures. One hundred and

ninety-two seeds from each of these treatments were planted in eight flats February 27, and measurements were made of the seedlings on March 4, 6, and 8. The measurements are given in table 11. Errors were calculated for only the last two measurements, when germination was complete. Delayed death was evident at the third measurement, and the number of living plants was recorded March 14.

TABLE 11.—*Effect of seed temperature during X-raying on size of seedlings and number of survivors, March 1940*

Treatment		Total seedlings			Mean height of seedlings			Seedlings surviving
Seed temperature (° C.) at exposure to X-rays	X-ray dosage	Mar. 4	Mar. 6	Mar. 8	Mar. 4	Mar. 6[1]	Mar. 8[2]	Mar. 14
	Kr.	*No.*	*No.*	*No.*	*Mm.*	*Mm.*	*Mm.*	*No.*
−187	32.7	186	188	188	14.52	16.77	16.97	0
−62	32.7	187	188	188	14.32	16.09	16.02	0
0	32.7	187	188	188	13.99	15.43	15.36	0
25	32.7	189	189	189	13.65	15.31	15.07	0
50	32.7	181	184	184	19.69	28.93	31.99	50
66	34.3	187	187	187	18.58	25.25	26.37	17

[1] Differences to be significant must exceed 1.66 mm.
[2] Differences to be significant must exceed 1.78 mm.

The seedlings from the 50° and 66° C. treatments were significantly larger than those from the lower temperatures. In this experiment, as in experiment 2, the seeds treated at 66° produced smaller seedlings than those treated at 50°. The difference in size of seedlings between the 50° and 66° lots may be the result of an overcorrection for back-scattering. However, it corresponds to that found in experiment 2, where the X-ray dosages were not modified to correct for back-scattering.

As in experiment 6, the seeds treated at 50° C. were slower in germinating and had a lower percentage of germination than those of the other treatments. In this experiment, however, it cannot be demonstrated that this peculiar behavior of seeds subjected to X-raying at 50° is other than a caprice of chance.

It is worthy of observation that although the plants in this experiment attained a size comparable to those in experiment 6, indicating closely similar growing conditions, the only surviving seedlings were found in the two high-temperature treatments.

EXPERIMENT 8, SMALL-TEMPERATURE-VARIATION TESTS, MARCH 1940

For the foregoing experiments the relative biological response was found to be reproducible within the temperature range −187° to approximately 50° C.; however, some uncertainty was found to exist for the higher temperatures. In experiment 2 the X-ray sensitivity appears slightly less for the 60° value than for the 50° point; in this case a hot-water bath was used for heating the seeds. However, as reported in experiments 6 and 7, the seeds kept at 66° showed greater injury than those held at 50°. In these later experiments, the temperature-control oven was used for the 66° value while the hot-water bath was used for the 50° point. Seeds in the oven rested on a thin paper support with practically no back-scattering, while the

seeds in the water bath received the back-scattering arising from the metal boat in which they were placed. Since tests made with the open ionization chamber showed that the amount of back-scattering from the metal boat was about 8 percent of the primary beam intensity, the dosage given to the seeds in the oven was increased by about 5 percent to compensate for the absence of back-scattering. The seeds were lying on the metal support with their germ sides up; thus a certain amount of the back-scattered radiation was probably absorbed in the endosperm so that the 5-percent factor may be an overcorrection, which would explain the apparent increase of X-ray sensitivity at 66°.

In order to test further the variations in sensitivity at these high temperatures, the oven was used for both the 50° and 66° C. treatments. By this means differences in back-scattering were avoided and the temperatures were more accurately controlled. Also, lots of seed were included to determine the effect of a dosage variation of about 5 percent. Three lots of 192 seeds each were treated, 2 lots at 66°, 1 of which was irradiated at a dosage of 35 kr., and 1 at a dosage of 36.7 kr. The third lot was treated at 51° with a dosage of 35 kr. The 3 lots of seeds were planted in the greenhouse on March 8 in 4 flats in randomized 3-row blocks. Measurements were made on March 14, 16, and 18, but only the results of the last measurement, at which time germination was complete and delayed death had not become apparent, are shown in table 12. The number of living plants was recorded March 23.

TABLE 12.—*Effect of seed temperature during X-raying on size of seedlings and number of survivors March 1940*

Treatment		Total seedlings [1]	Mean height of seedlings [2]	Seedlings living Mar. 23
Seed temperature (° C.) at exposure to X-rays	X-ray dosage			
	Kr.	*Number*	*Millimeters*	*Number*
51	35	189	26.97	32
66	35	184	29.34	63
66	36.7	183	25.04	36

[1] 192 seeds in each treatment.
[2] Standard error equals ±0.98.

In experiment 8, seeds X-rayed at 35 kr. showed a lower sensitivity at 66° than at 51° C. The experiment, however, was not efficient enough to establish differences of less than 10 percent in mean size. Limiting the comparison to the two temperatures having 35 kr. irradiation, it is seen that 66° has not exceeded the optimum for reducing sensitivity to X-radiation. Although the difference in mean height (2.37 mm.) is not statistically significant, the fact that there were twice as many survivors at the higher temperature may be considered strong evidence that the differences in seedling height are ascribable to the temperatures. The increase of 1.7 kr. of radiation produced a statistically significant reduction in seedling height.

DISCUSSION

The data on the percentages of survivors and on mean heights given in experiments 5, 6, and 7 can be consolidated to give values

showing more clearly the temperature effects within the range —187° to about 50° C. In figure 2 is shown the percentage survival as a function of the temperature and in figure 3 are shown the mean

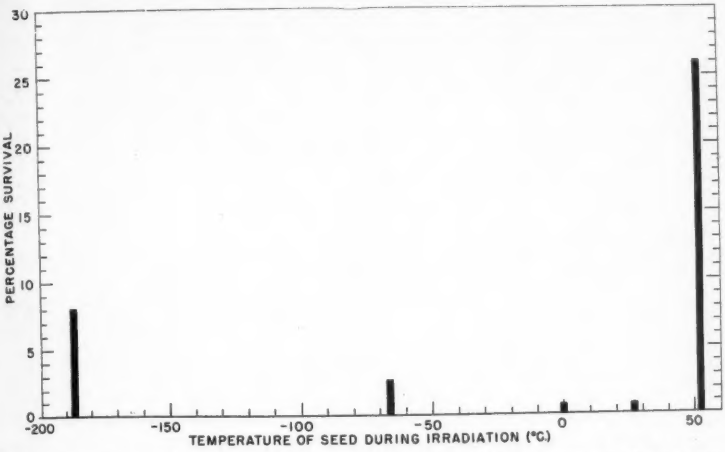

FIGURE 2.—Percentage survival as a function of seed temperature during irradiation (experiments 5, 6, and 7).

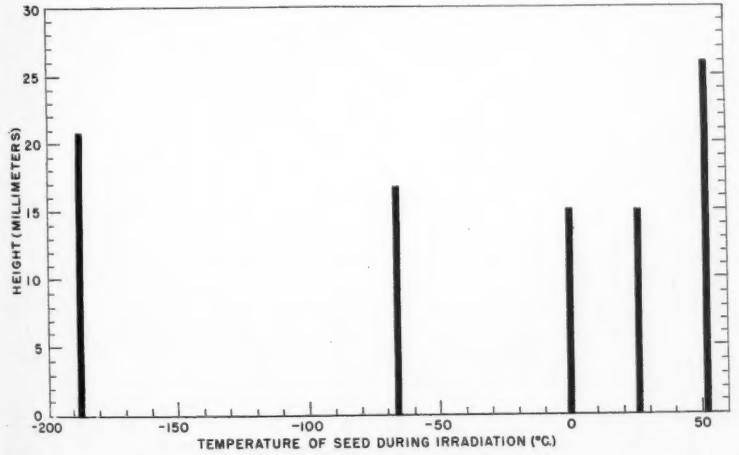

FIGURE 3.—Mean heights of plants as a function of seed temperature during irradiation (experiments 5, 6, and 7).

heights. The greatest survival is obtained at about 50°, and the lowest survival in the 0° to room-temperature range. In deriving the means it was necessary to weight each mean proportional to the mean height of its particular group. These diagrams suggest the possibility that quite different factors are influencing X-ray sensitivity. One set appears to predominate below about 27°, where the relation-

ship of temperature and X-ray sensitivity is essentially linear. Another set, which greatly reduces X-ray sensitivity, comes into operation above about 27°. The 66° value was not included because of the uncertainty mentioned above concerning the back-scattered radiation. However, on the basis of the data obtained in experiments 2 and 8, where no back-scattering factor entered, the 66° heat treatment resulted in a further reduction in the X-ray sensitivity.

The experiments described above show repeatedly and in a consistent manner that the X-ray sensitivity of maize seeds depends upon the temperature during the time of irradiation. It has also been demonstrated that the X-ray sensitivity is greatest in the range of room temperature to 0° C. An increase of temperature to 50°, or a decrease of temperature to −187° will in either case result in a lowering of the X-ray sensitivity.

A purely physical interpretation (*6*) of the mechanism responsible for delayed death showed, on the basis of the so-called "hit" theory, that the seed did not contain a single sensitive volume of dimensions required by the theory. It is possible, however, that the seed embryo contains several rather widely separated sensitive volumes, each one of which must suffer a number of hits by primary or secondary electrons to produce delayed death. Death of the plant may result, therefore, from an interaction between the sensitive volumes and the remainder of the material, as well as from interactions existing between themselves. Temperature alone obviously will not change the rate of quanta absorption or the number of hits per unit volume; however, the transportation mechanisms between various portions of the seed will be temperature-dependent. The observed decreased sensitivity at the low temperature may be due to a slowing up of the cell mechanism for dissemination of energy received from the primary electrons. This would result in a greater localization of the absorbed energy so that the activated sensitive volumes would be less effective in killing the plant. Although a theory of this kind may hold as an explanation of the reduced sensitivity at low temperatures, it fails to explain the response observed at high temperatures.

A conclusion that the ultimate cause of delayed death is to be found in the derangement of chromosomes with consequent breakdown in mitosis restricts the factors contributing to sensitivity to those which in some manner will affect the behavior of chromosomes. In the present experiments these factors must be present during exposure to X-rays. In resting seeds where no cell divisions are taking place, the chromosomes are not found as separate bodies and, although the chromatin appears as disconnected dots, these dots may be organized in an extended thread form. In this condition the chromosomes are subject to breakage. If the cause of seedling demise is due to physical injury of the chromosomes, then the external factors which reduce sensitivity to X-rays must operate in such a way as to protect the chromatin organization from these injuries or to heal the injuries after they occur.

Other investigators dealing with temperature-dependent sensitivities have attributed the reduced sensitivity at high temperatures to recovery. For example, Saks and Enzmann (*8*) investigated the effect of temperatures within the range 3° to 38° C. on chromosome breakage in *Tradescantia*. In their work flower buds were X-rayed at several temperatures and a cytological examination was made of the

chromosomes. It was found that with increase in temperature there was an apparent decrease in broken chromosomes. They attributed their failure to observe breakages at the higher temperatures to recovery. They assumed that breakages took place equally at all temperatures but at the higher temperatures the broken ends reunited.

Fabergé (*5*), also working with *Tradescantia*, has essentially confirmed the results of Saks and Enzmann by observing a reduction in the number of chromosome breakages when flower buds were X-rayed at 30° as compared with 15° C.

Spear (*9*) found that in animal-tissue cultures exposed to gamma rays at 37° and at 1° C. the reduction in mitosis was identical, but that recovery required a much longer time when cultures were irradiated at the lower temperature.

Cook (*2*), on the other hand, obtained greater recovery of one-cell *Ascaris* eggs from 5 kr. by holding them at 5° C. after treatment than at 25°. Delay in cleavage, however, was found to be temperature-independent. These recovery effects in tissue culture can hardly be applied to dry seeds, where there is no active cell division. It seems more likely that chromosome breakages are not the most important factor in the present experiments, since no obvious mechanism is apparent for reuniting the ends in the resting seeds.

No attempt has been made in the current experiments to establish the regularity of chromosome behavior as the result of high or low treatment temperatures in all the plants surviving these dosages. However, pollen mother cells of several such plants have been examined by Longley and found to be normal. Other plants have been transplanted and grown to maturity in the field without showing abnormal behavior. They produced viable pollen without excessive quantities of sterile grains, and they matured seed when self-pollinated and functioned as male parents on non-X-rayed plants. This behavior is fairly satisfactory evidence that these plants, and presumably, therefore, all the seedlings that survived, had normal cytological development. The only visible evidence of abnormality was a very fine striation in the leaves, found invariably on all the seedlings from X-rayed seed in these experiments.

Apparently the temperatures to be effective must prevail during irradiation. The single observation reported here (experiment 5) where the X-rayed seeds were subjected to high temperature following irradiation showed no significant beneficial effect of postirradiation temperature. Furthermore, when seeds were subjected to high temperature before irradiation (experiment 3) they were clearly more, rather than less, sensitive to X-rays. These results with anteirradiation and postirradiation temperatures are admittedly limited and are noted here as merely suggestive. Their bearing on the subject of the nature of X-ray injury can await a more adequate demonstration that temperatures at other than the irradiation period are ineffective. Experiments are now in progress to test this question.

SUMMARY

Certain organisms X-rayed in the resting stage respond to heavy dosages by dying soon after an apparently normal early growth. In the case of air-dry maize seeds this phenomenon, which has been called delayed death, is characterized by the death of the seedling after normal germination. Repeated experiments show in a consistent

manner that the X-ray sensitivity of air-dry (8-percent moisture) seeds of maize depends upon their temperature during the time of irradiation.

The temperatures used ranged from −187° to 66° C. and the X-ray dosages from 30 to 45 kr. Heights of plants and the survival ratios were used for determining X-ray sensitivity.

It was found that maximum sensitivity occurred for the temperature range of 0° C. to room temperature. Either an increase or a decrease of the seed temperature from these values resulted in a reduction of the X-ray sensitivity.

X-ray dosages of 30 to 45 kr. are known to cause derangements of the chromosomes, but since the material used in these experiments was in the resting stage it is believed that the observed decrease in sensitivity at the extreme temperatures cannot be explained as recovery from injury to the chromosomes. Further questions concerning the interpretation of these results are discussed.

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PRODUCTION OF HEAT AND OVULATION IN THE ANESTROUS EWE[1]

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INTRODUCTION

In most sections of the United States the ewes of most breeds of sheep will breed only during the fall and winter months. Because of this fact, the majority of lambs are born in the spring and marketed in the fall. Lambs going to market during the spring and early summer generally bring a higher price, but for their production the ewes must be bred during the spring months. A sure but economical means of causing ewes to breed at this season would be of decided value to the sheep industry. Experimental attempts to produce heat and ovulation in the anestrous ewe by injections of endocrine substances are reported in this article.

Ewes may show signs of estrus without ovulating. It is necessary, therefore, to have some means of determining whether ovulation occurs. An operation permitting inspection of the ovaries has been employed by some investigators, but this procedure has very evident disadvantages. Suggestions have been made that ovulation could be detected by changes in the type of vaginal secretion, and a review of the vaginal smear records of the treated ewes has, therefore, been included in the study.

REVIEW OF LITERATURE

Cole and Miller (*3*)[2] and McKenzie and Terrill (*6*) brought ewes into estrus during anestrum by injections of estrongenic substances. The former used estrogenic hormone from mare urine. McKenzie and Terrill injected Progynon-B and were most successful with the use of 900 to 1,500 rat units.

The induction of ovulation accompanied by estrus has been reported by Cole and Miller (*2*, *3*). The gonadotropic hormone of pregnant-mare serum was given in doses of 100 rat units spaced at 17-day intervals. Simultaneous injections of pregnant-mare serum and estrogen commonly resulted in estrus without ovulation. Injections of pregnant-mare serum followed by injections of estrogen 2 days later commonly resulted in ovulation without estrus.

McKenzie and Terrill (*6*) obtained ovulation without estrus in 5 of 11 anestrual ewes injected with 300 to 400 rat units of pregnant-mare serum.

Casida (*1*) has induced ovulation in 3- to 5-month-old lambs by injecting, subcutaneously, small doses of a pituitary extract, prepared as a partially purified follicle-stimulator.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 625.

Changes in the vaginal smear have been reported by Hawkins and Darlow (*5*), Grant (*4*), Cole and Miller (*3*), Polovzeva and Fomenko (*7*), Richter and Rittau (*8*), and McKenzie and Terrill (*6*). The findings are not entirely in accord. Grant (*4*) and Richter and Rittau (*8*) doubt the reliability of the vaginal smear as an indicator of the stages of the estrual cycle. Cole and Miller (*3*), however, believe that the presence of large quantities of transparent, watery mucus characterizes early estrus; and a copious, dry, cheesy smear consisting chiefly of large squamous epithelial cells characterizes metestrum. Polovzeva and Fomenko (*7*) reported that the vaginal smear was a reliable indicator of ovulation, ovulation usually occurring after the appearance of cornified cells and before the appearance of leucocytes in the vaginal smear.

EXPERIMENTAL PROCEDURE

EXPERIMENTAL ANIMALS

The sheep used in this study included 30 yearling ewes, which were selected from two groups of feeder lambs, near Madison, Wis., in the spring of 1938. These ewes were of western origin (predominantly fine wool) and averaged about 85 pounds in weight at the time of purchase. They were kept in a barn during the entire experimental period, except for brief exercise periods when they were turned into a dry lot. Alfalfa hay consumed ad libitum was the sole feed during the study.

VAGINAL SMEARS

Vaginal smears were taken from each ewe daily, except for a short period during the summer. In making these smears a speculum, made of 12-mm. glass tubing approximately 22 cm. long, and an 8-mm. glass rod, about 12 cm. longer than the speculum, were used. The ewe to be smeared was placed in a stanchion, the glass speculum inserted into the vagina and pushed forward and upward until the cervix was reached. The rod was then inserted through the speculum, the speculum withdrawn 1 to 2 inches, and the smear taken by rotating the inserted end of the rod on the exposed surface of the anterior vagina. The rod and speculum were removed after the upper end of the rod had been pulled back into the speculum to prevent contamination from any other region of the vagina at the time of withdrawal.

The consistency of the mucus found on the rod was noted and recorded as "thin," "thick," or "cheesy." If no mucus was present the smear was recorded as "clear." The smeared end of the rod was then rotated in a drop of water or eosin solution on a slide and this slide was examined under the low-power objective of a microscope. No actual counts of the various cell types found were made, but estimates of the numbers of the large, round, nucleated epithelial cells, the cornified epithelial cells, or "scales," and leucocytes were made. Each type of cell was given a value of "none," "very few," "few," "several," "many," or "very many."

INDUCTION OF HEAT AND OVULATION

All the ewes were given endocrine injections to produce heat or heat and ovulation. Small groups, commonly four, were injected at different times during May, June, and July. All the ewes were

"teased" daily with aproned rams to determine if any were in heat (receptive to the male). The uninjected individuals at any treatment period thus served as controls for the appearance of heat in similar but untreated ewes. At the end of the period of experimental treatment, the ewes were spayed and the ovarian response to the treatment was checked at the time of removal of the ovaries.

ENDOCRINE SUBSTANCES USED

Progynon–B (Schering Corporation) is the benzoic-acid ester of dihydrofollicular hormone. This material was injected subcutaneously, commonly in the shoulder region. The pregnant-mare serum used was prepared by acetone precipitation and desiccation.[3] Immature-rat-ovary assays gave average weights of 36 mg. (4 rats) and 126 mg. (4 rats) from injections of 25 and 50 mg., respectively, of the preparation. Twenty-five mg. were then assumed to be equal to 1 rat unit. The material was injected subcutaneously as an aqueous suspension into the shoulder and flank regions. Sheep anterior pituitary extract was obtained from the zoological laboratory of the University of Wisconsin and was prepared from acetone-desiccated powder by extracting three times with water—ratio of 1 gm. of powder to 10 cc. of water. The aqueous extract was precipitated with acetone, recovered by centrifuging, and dried with acetone. Assays made by the zoological laboratory gave average ovarian weights as follows: 400 milligram-equivalents of the desiccated gland (12 rats), 115 mg.; 200 milligram-equivalents (12 rats), 91 mg.; 100 milligrams-equivalents (15 rats), 65 mg.; and 50 milligram-equivalents (12 rats), 41 mg.

TABLE 1.—*Results of injections of endocrine substances upon the production of heat and ovulation*

Number of ewes	Treatment [1]	Ewes showing heat	Ewes ovulating	Average number of ovulations in ewes that ovulated
8	Single injection of 100 rat units of pregnant-mare serum	0	7	1.56
4	100 rat units of pregnant-mare serum and 1,000 rat units of Progynon–B simultaneously.	1	2	1.00
2	100 rat units of pregnant-mare serum followed on the 1st, 2d, and 3d days by 2 injections of 500 rat units and 1 injection of 1,000 rat units of Progynon–B.	1	2	1.00
1	100 rat units of pregnant-mare serum followed on the 3d and 4th days by 2 injections of 1,000 rat units of Progynon–B.	0	1	1.00
1	100 rat units of pregnant-mare serum followed on the 3d day by a single injection of 1,000 rat units of Progynon–B.	0	0	0
3	1,000 rat units of Progynon–B in single injections	2	0	0
3	2,000 rat units of Progynon–B in single injections	2	0	0
2	2,000 rat units of Progynon–B in 3 successive daily injections of 500, 500, and 1,000 rat units.	1	0	0
4	2.5 gram-equivalents of sheep anterior pituitary extract given in 4 daily 0.5 gram-equivalent subcutaneous injections, followed by 0.5 gram-equivalent intravenous injection on the 5th day.	0	4	6.25
1	100 rat units of pregnant-mare serum and 1 gram-equivalent sheep anterior pituitary extract simultaneously.	0	1	1.00
1	100 rat units of pregnant-mare serum, 1 gram-equivalent sheep anterior pituitary extract, and 1,000 rat units of Progynon–B simultaneously.	0	1	3.00

[1] Unless otherwise stated, injections were made subcutaneously.

[3] Grateful acknowledgment is made to Dr. W. H. McShan for the preparation of the pregnant-mare serum and the sheep anterior pituitary extract, and to Dr. R. K. Meyer for the assay of these materials.

RESULTS

Ovulation was produced in seven of the eight ewes receiving 100 rat units of pregnant-mare serum (table 1). Five of the ewes had single ovluations, one had two ovulations, and another four. The time of ovulation could not be definitely ascertained, but in the cases of two ewes ovulation had occurred before the spaying operations, which were made on the fourth day following the injection of pregnant-mare serum. None of the eight ewes receiving pregnant-mare serum alone showed the behavior of estrus.

Two of the four ewes that received pregnant-mare serum and Progynon-B, simultaneously, ovulated. Both cases were single ovu-

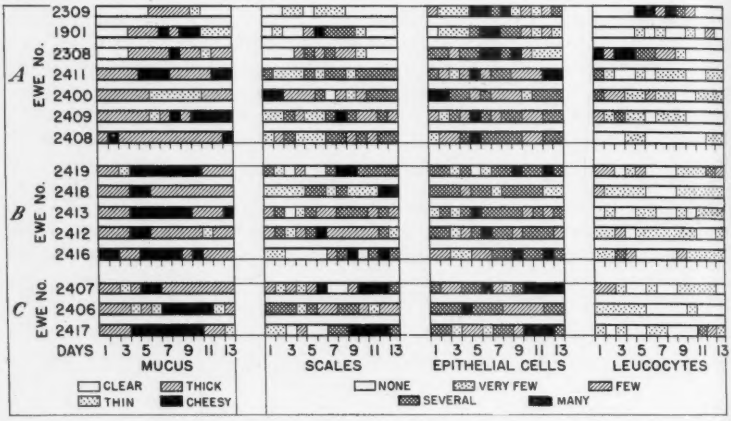

FIGURE 1.—Vaginal smear records of 15 injected ewes: *A*, 7 ewes given injections of 100 rat units of pregnant-mare serum on the second day, followed by ovulation; *B*, 5 ewes given injections of Progynon-B on the second day, estrus produced; *C*, 3 ewes given injections of Progynon-B on the second day, no estrus produced.

lations and ovulation was not accompanied by heat in either ewe. One of the two ewes not ovulating showed signs of heat.

Three of the four ewes that received pregnant-mare serum followed by Progynon-B ovulated. One of these came into heat 5 days after the injection of pregnant-mare serum (the time relation between heat and ovulation is not known). She was given 500 rat units of Progynon-B on the first and second day and 1,000 rat units on the third day following the injection of pregnant-mare serum. The fourth ewe neither ovulated nor came into heat.

Five of the eight ewes receiving Progynon-B alone came into heat. The length of the estrual period was impossible to determine in some cases as the ewes were spayed while still in heat. In the cases of three ewes which were spayed after going out of heat, two had estrual periods of 1 day and another of 2 days in length.

All four of the ewes receiving sheep anterior pituitary extract ovulated. The number of ovulations per ewe ranged from 2 to 13. None of the ewes showed any signs of estrus. Ovulation occurred in the ewe receiving pregnant-mare serum and sheep anterior pituitary

extract and in the one receiving pregnant-mare serum, sheep anterior pituitary extract, and Progynon-B, but neither ewe came into heat. No evidences of heat were seen in any of the ewes at any time other than following some endocrine treatment.

Vaginal smear records from 15 of the ewes are presented in Figure 1. Seven of these ewes ovulated following injections of pregnant-mare serum. The other eight were given Progynon-B. Five of these came into heat, while three showed no signs of estrus. Considerable variation was shown among the ewes, but the same general trends are indicated in the number of the different cell types, as well as in the consistency of the mucus in the three groups of ewes following the injections.

The most consistent changes are seen in the character of the mucus. These changes are most striking in the Progynon-B-treated ewes. The smears of these ewes became "cheesy" on the second to third day after the injection. The smear of the ovulating ewes tended to follow the same trend, as indicated by an increase in thickness after injections of pregnant-mare serum.

Changes in number of "scales" and epithelial cells appearing in the smears of the ewes following injections again show much individual variation within treatment groups and little, if any, difference between groups. Trends again are indicated and an increased number of both cell types were commonly found following the injections in all three groups. Leucocytes did not appear with sufficient regularity in the smears of the three groups to describe any general trend in their number following injections of either pregnant-mare serum or Progynon-B.

DISCUSSION

Pregnant-mare serum, given in single subcutaneous injections of 100 rat units, produced ovulation rather uniformly in the anestrual ewes, but there were no signs of heat accompanying ovulation. It would, therefore, be necessary to force-mate or artificially inseminate such ewes at about the time of expected ovulation in order to have fertilization. (This is assuming that the ova are potentially fertile.) The situation is somewhat analogous to the "quiet" ovulations reported by Roux (*9*), who found ovulation without estrus occurring in Merino ewes during the anestrous period and particularly at the beginning and end of the breeding season.

The smallness of the dosage apparently does not explain the failure of these ewes to come into heat. Cole and Miller (*2*), (*3*) and McKenzie and Terrill (*6*) have given pregnant-mare serum at a level several times as high as that necessary to produce ovulation without causing the ewes to come into heat. Cole and Miller (*3*), however, have found that doses of 100 rat units spaced at 17-day intervals will produce both heat and ovulation. The latter procedure was not tried in this study.

The results from injections of anterior pituitary extract are similar to those obtained from the use of the pregnant-mare serum. All of the pituitary-treated ewes ovulated without a sign of estrus. Total doses given were 2.5 gm. (100 rat units) of pregnant-mare serum in single subcutaneous injections and 2.5 gram-equivalents of anterior pituitary powder in four daily subcutaneous injections of 0.5 gram-equivalent followed by an intravenous injection of the same amount.

Assay comparisons of sheep pituitary extract and of pregnant-mare serum may not be warranted because of the different nature of the assay curves for the two preparations. The ovarian weights, however, for the rats receiving 25 mg. of pregnant-mare serum were approximately equal to those for rats receiving 50 mg.-equivalents of anterior pituitary, and if this be accepted for comparison, the sheep treated with pregnant-mare serum received approximately twice as many rat units of hormone as those receiving pituitary. In spite of the greater potency of the pregnant-mare serum indicated by these assays, pituitary extract administered in total doses similar to the total doses of the serum gave a much higher ovulation rate. It is possible that dividing the total dose of pituitary extract into four subcutaneous and one intravenous injection may have been responsible for the increased ovulation rate. It is also possible that the ewe gives a greater response, per rat unit, to pituitary extract than to pregnant-mare serum. As the two materials were not injected in a similar manner, the correctness of these assumptions cannot be checked. Similar results have been obtained by Casida (*1a*), however, when the two hormone preparations were injected alike into cattle.

If a small amount of estrogen is responsible for the failure of the ovulating ewe to come into heat, it would seem reasonable to expect that supplemental estrogenic injections would be of value. However, as has been previously reported by Cole and Miller (*3*), combinations of both pregnant-mare serum and estrogens are usually not successful in producing both heat and ovulation. Although the number of ewes given this type of treatment in the present study is small, the results also indicate the difficulty in producing both heat and ovulation with pregnant-mare serum and Progynon-B.

The production of heat in the anestrual ewes by the use of Progynon-B was not uniformly successful in this study. The number of ewes treated was small, and there is little to indicate whether the higher or lower doses were more successful in producing estrus. These results are not surprising in view of the results of McKenzie and Terrill (*6*), who reported producing estrus in only 44 percent of 52 ewes given single injections of 800 rat units of Progynon-B.

The changes in the vaginal smear during artificially induced estrus or ovulation are in general similar to those described at natural estrus. No marked difference was shown between the smears of ewes which ovulated without showing signs of estrus and those of ewes which came into heat but did not ovulate. It would be impossible with the technique used in this study to determine by means of the vaginal smear whether ewes showing signs of heat had ovulated or not. It would appear that the changes in the vaginal smear are produced primarily by estrogenic substances and influenced little, if at all, by the hormone of the corpus luteum.

SUMMARY AND CONCLUSIONS

Thirty ewes were included in this study. Injections of pregnant-mare serum, sheep anterior pituitary extract, and Progynon-B, singly or in combination, were given to all of these ewes. Occurrence of heat was checked by the use of "teaser" rams and the ovarian stimulation was noted at the time of spaying.

Ovulation without heat was produced regularly by the use of either pregnant-mare serum or anterior pituitary extract. The number of ovulations per ewe was near normal in the ewes given pregnant-mare serum, but ranged from 2 to 13 in ewes given sheep anterior pituitary powder.

Progynon-B produced heat in five out of eight treated ewes.

Combinations of pregnant-mare serum and Progynon-B were generally unsuccessful in producing both heat and ovulation.

Vaginal smear records indicated no difference between the smears of ewes in which ovulation was induced without heat and the smears of ewes in which estrus was induced without ovulation.

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THE CHEMICAL COMPOSITION OF FOREST FRUITS AND NUTS FROM PENNSYLVANIA [1]

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INTRODUCTION

In the interest of wildlife conservation it is important to know the nutritive values of the food products of the forest, especially those which are available for the building up of nutritive reserves during the fall, to carry the wildlife over the critical winter period. Only after the various mast and browse foods have been evaluated is it possible, by proper forest management, to encourage the growth of the more desirable species. These are not necessarily the most nutritious ones, however, but rather are those which combine availability, productivity, nutritive value, and a capacity to resist decay. Upon this knowledge, and that of the food habits of the animals of the forest, wildlife management must to a large extent depend.

This paper seeks to represent, by means of chemical analysis, the nutritive value of 35 mast products from central Pennsylvania.

In making this study it was recognized that the possibilities of representing food values by chemical analysis are limited, and that the most significant information on the subject can be had only as a result of the use of the food products by animals. The mast foods are so diverse in character, however, some being concentrated foods capable of furnishing large parts of animals' diets, while many more contain little nutriment, and normally serve only as minor components of highly complex diets, that the only practicable method of conducting a general survey of the subject was by chemical analysis.

METHODS

Beginning with the conventional feed analysis in terms of moisture, total nitrogen, ether extract, crude fiber, ash, and nitrogen-free extract, additional determinations of tannin, cellulose, lignin, available nitrogen, calcium, magnesium, and phosphorus were made.[2] Record was made (table 1) of the date and the approximate location of collection when this latter information was available. Three of the samples, namely, the nuts of the Italian chestnut, shellbark hickory, and hazel, were purchased in the market. The Italian chestnut was included with the idea that the analysis of this product might serve approximately to represent the American chestnut if and when it shall again attain a significant prominence as a mast food.

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[2] The materials analyzed were collected by L. J. Bennett, P. F. English, T. Kuhn, and R. McCain.

TABLE 1.—*Name, date of collection, source, and portion analyzed of the fruit and nut products examined*

FRUITS

Common name [1]	Scientific name [1]	Date collected	Source in Pennsylvania	Portion analyzed
Apple, crab	*Malus coronaria*	Sept. 29, 1938	Huntingdon County	Whole fruit.
Bittersweet	*Solanum dulcamara*	Oct. 13, 1938	Centre County	Fruit, without calix.
Blackberry	*Rubus occidentalis*	Aug. 2, 1939	do	Whole berries.
Blackberry, Bailey's	*Rubus baileyanus*	Aug. 1, 1939	do	Do.
Blackhaw	*Viburnum prunifolium*	Sept. 29, 1938	do	Whole berries, unripe.
Blueberry	*Vaccinium sp.*	Aug. 1, 1939	do	Whole berries.
Cherry, wild	*Prunus virginiana*	Sept. 29, 1938	do	Whole fruit (1) and seeds (2).
Chokeberry, black	*Aronia melanocarpa*	July 31, 1939	do	Whole berries.
Chokeberry, red	*Aronia arbutifolia*	Oct. 18, 1939	do	Do.
Cucumbertree	*Magnolia acuminata*	Sept. 23, 1938	Warren County	Whole fruit, three-fourths ripe.
Deerberry	*Accinium stamineum*	Aug. 29, 1939	Centre County	Whole fruit, unripe.
Dogwood, panicled	*Cornus femina*	Oct. 13, 1938	do	Whole berries.
Dogwood, red-osier	*Cornus stolonifera*	Sept. 25, 1939	do	Whole berries, half ripe.
Elder, American	*Sambucus canadensis*	Sept. 26, 1939	do	Whole berries.
Grape, frost	*Vitis cordiofolia*	Oct. 13, 1938	Huntingdon County	Whole fruit.
Hackberry	*Celtis occidentalis*	Nov. 2, 1939	Centre County	Whole berries.
Hawthorn, cockspur	*Crataegus crus-galli*	Oct. 13, 1938	do	Whole fruit.
Juneberry	*Amelanchier canadensis*	July 7, 1939	do	Whole berries.
Mountain-ash, American	*Sorbus americana*	Sept. 29 1938	Huntingdon County	Do.
Mountain-holly	*Nemopanthus mucronata*	Aug. 10, 1939	Centre County	Do.
Nannyberry	*Viburnum lentago*	Sept. 25, 1939	do	Whole berries, nearly ripe.
Spicebush	*Benzoin aestivale*	Sept. 29, 1938	Huntingdon County	Fleshy part (1), and seeds (2).
Sumac, smooth upland	*Rhus glabra*	Sept. 29, 1938	Centre County	Whole berries.
Sumac, staghorn	*Rhus hirta*	Oct. 18, 1939	do	Do.
Winterberry, Virginia	*Ilex verticillata*	Oct. 17, 1939	do	Do.

NUTS

Buckeye, fetid	*Aesculus glabra*	Sept. 25, 1939	Centre County	Kernels.
Chestnut, Italian	*Castenea vulgaris*		Open market	Do.
Hazelnut	*Corylus americana*	Sept. 3, 1938	Centre County	Do.
Hickory, shell-bark	*Hicoria ovata*		Open market	Do.
Oak, red	*Quercus rubra*	Oct. 20, 1938	Warren County	Kernels, with integument.
Oak, rock chestnut	*Quercus prinus*	Oct. 7, 1938	Huntingdon County	Do.
Oak, scrub	*Quercus ilicifolia*	Sept. 29, 1938	do	Do.
Oak, scrub chestnut	*Quercus prinoides*	Sept. 25, 1939	Centre County	Do.
Oak, white	*Quercus alba*	Oct. 13, 1938	do	Do.
Walnut, black	*Juglans nigra*		Open market	Kernels.

[1] Authority: BRITTON, NATHANIEL LORD, and BROWN, ADDISON. AN ILLUSTRATED FLORA OF THE NORTHEASTERN UNITED STATES, CANADA AND THE BRITISH POSSESSIONS, FROM NEWFOUNDLAND TO THE PARALLEL OF THE SOUTHERN BOUNDARY OF VIRGINIA AND FROM THE ATLANTIC OCEAN WESTWARD TO THE 102D MERIDIAN. Ed. 2, rev. and enl., 3 v., illus. 1936. [N. Y. Bot. Gard.]

As the materials were received they were either immediately prepared for analysis or were stored for a limited time in sealed containers in refrigerating rooms. The fruits and berries were stored at approximately 40° F. and the nuts at temperatures below freezing.

In preparation for preliminary drying the fleshy fruits were sliced, and the nut kernels were cut into small pieces. The former were then dried in an air oven at approximately 50° C., and the latter in a vacuum oven at room temperature with reduced air pressure.

In the case of the wild cherry, separate analyses were made of the whole berries and of the seeds; and in the case of spicebush berries separate analyses were made of the outer fleshy integument and the seeds. The figures for the pulp and skin of the wild cherry, and for the whole spicebush berry, were calculated from the parts analyzed.

The oven-dry materials were transferred to a screened cabinet in which they were allowed to come to equilibrium with the moisture of the air. After 7 to 10 days they were weighed, and the loss of moisture from the fresh to the air-dry state was recorded.

The air-dry samples were ground in a meat chopper, and then extracted for 48 hours, the oily products (nuts, spicebush berry, etc.) with ether, and the sugary substances (blueberry, blackberry, etc.) with 95-percent ethyl alcohol. The air-dry residues were then ground in a micro Wiley mill, to pass a 20-mesh sieve, after which they were recombined, quantitatively, with their respective extracts.

The recombined materials were again dried in the air oven to remove the solvent, and were then rubbed through a 20-mesh sieve. Finally they were placed in the screened cabinet, and allowed once more to come into equilibrium with the moisture of the air; and after 7 to 10 days were bottled and sealed in readiness for analysis.

The methods of the Association of Official Agricultural Chemists (*1*) [3] were followed in the determination of moisture, total nitrogen, ether extract, crude fiber, total ash, nitrogen-free extract, tannin, calcium, magnesium, and phosphorus.

In determining moisture the samples were dried for 21 days in vacuum desiccators, without heat, in the presence of sulfuric acid. The samples thus dried were used in the determination of ether extract.

In employing the Kjeldahl-Gunning-Arnold method for total nitrogen, 4-percent boric acid was used as the receiving liquid, as proposed by Winkler (*7*).

Available protein was determined by the method of Horwitt, Cowgill, and Mendel (*4*), but this procedure was found not to be especially well adapted to the routine analysis of mast products because of excessive frothing during digestion.

Lignin was determined by the method of Ross and Hill (*6*) as modified first by Crampton and Maynard (*2*), and later by Crampton [4] by the use of Whatman, No. 50, acid-hardened filter paper in place of bolting cloth, and of preignited Celite Analytical Filter-Aid in place of the granulating agent consisting of chloroform and acetic acid. Cellulose was determined by the method of Kirschner and Hanak (*5*) as applied by Crampton and Maynard (*2*) to feeding stuffs.

ANALYTICAL RESULTS

The results of the chemical analyses of the mast products are given in table 2 expressed on the dry basis, and in table 3 on the fresh basis. Comments will be made on the former alone, since, from the nutritive point of view, the moisture contained in the materials is simply a variable diluent.

[3] Italic numbers in parentheses refer to Literature Cited, p. 635.
[4] Unpublished data.

The sum of the values for lignin and cellulose was invariably higher (124 to 543 percent) than the single figure for crude fiber. This may be due to a loss of cellulose during the acid and alkali digestions involved in the determination of crude fiber; or the values for lignin and cellulose may conceivably be improperly high as a result of undetermined impurities.

The content of available protein was less than the crude protein in all products, indicating the presence of some fat-soluble, nitrate, amide or ammonia nitrogen. The error of regarding nonprotein nitrogen as protein nitrogen is of less significance when the foods are used by ruminants than when they are used by nonruminants, because the former have the capacity to utilize nitrogen from sources other than protein—presumably by virtue of the synthetic capacities of the bacteria of the rumen.

TABLE 2.—*Percentage analyses (dry basis) of the fruit and nut products examined*

FRUITS, DRY BASIS

Common name	Crude protein	Ether extract	Crude fiber	Total ash	N-free extract	Available protein	Lignin	Cellulose	Tannin	Calcium	Magnesium	Phosphorus
Apple, narrowleafed crab	5.56	6.19	16.95	3.16	68.14	4.00	11.89	15.48	4.71	0.02	0.09	0.17
Bittersweet	15.19	28.77	8.26	3.44	44.34	11.88	8.98	10.22	1.35	.27	.29	.36
Blackberry	8.19	7.58	21.43	3.12	59.68	6.63	31.73	13.52	1.72	.15	.14	.21
Blackberry, Bailey's	6.75	6.08	24.14	4.31	58.72	5.56	28.58	15.49	2.04	.12	.17	.12
Blackhaw	4.13	11.93	10.28	2.58	71.08	3.69	24.68	7.70	5.94	.05	.08	.13
Blueberry	4.19	3.80	9.67	1.44	80.90	2.75	13.85	7.97	1.28	.04	.07	.07
Cherry, wild, whole	6.75	6.26	20.85	2.84	63.30	5.75	18.65	12.92	.60	.16	.07	.16
Cherry, wild, seeds	13.13	15.70	50.85	1.57	18.75	11.25	38.29	25.28	.....	.18	.08	.19
Cherry, wild, pulp and skin	5.13	3.89	13.31	3.15	74.52	4.31	13.70	9.81	.75	.16	.07	.16
Chokeberry, black	5.00	3.44	12.56	2.71	76.29	4.19	39.80	9.80	3.78	.25	.12	.13
Chokeberry, red	5.25	3.80	9.24	2.52	79.19	4.38	36.13	8.48	7.31	.22	.21	.13
Cucumbertree	7.50	21.99	28.39	4.90	37.22	6.19	16.56	19.84	2.60	.23	.14	.20
Deerberry	3.75	5.45	11.17	1.62	78.01	2.50	11.46	8.57	1.71	.05	.05	.05
Dogwood, panicled	6.88	26.73	25.76	3.36	37.27	5.88	20.62	11.21	1.46	.21	.09	.15
Dogwood, red osier	6.94	12.02	26.42	3.40	51.22	5.56	27.12	12.01	1.58	.27	.19	.22
Elder, American	11.06	12.94	17.93	5.52	52.55	8.38	15.36	10.54	2.71	.13	.21	.36
Grape, frost	5.38	.87	13.43	2.75	77.57	4.19	14.50	7.45	1.99	.06	.08	.15
Hackberry	8.25	4.37	7.09	27.35	52.94	7.13	8.04	5.89	.82	12.42	.49	.22
Hawthorn, cockspur	2.81	3.29	32.83	3.69	57.38	2.56	20.44	23.32	3.39	.42	.10	.04
Juneberry	8.06	4.45	12.27	3.70	71.52	6.50	16.03	14.93	.41	.34	.21	.19
Mountain-ash, American	5.44	4.66	8.02	3.10	78.78	4.25	9.57	6.87	4.08	.10	.13	.16
Mountain holly	7.06	7.71	18.45	2.23	64.55	5.81	17.27	14.49	.96	.13	.13	.12
Nannyberry	4.13	8.88	7.18	2.06	77.75	3.63	32.32	6.69	1.57	.12	.04	.14
Spicebush, whole	11.94	50.73	5.23	5.74	26.36	9.94	4.04	5.98	1.35	.....	.13	.30
Spicebush, seeds	18.19	56.21	7.45	2.20	15.95	17.06	5.21	4.91	.58	.....	.16	.38
Spicebush, pulp and skin	8.56	47.82	4.05	7.62	31.95	6.13	3.41	6.54	1.75	.....	.11	.26
Sumac, smooth upland	4.13	11.23	34.90	2.45	47.29	3.56	22.56	27.99	6.89	.16	.07	.16
Sumac, staghorn	5.44	14.54	30.31	3.01	46.70	4.88	21.66	27.50	4.41	.30	.15	.25
Winterberry, Virginia	6.13	5.10	15.57	2.62	70.58	5.31	9.49	12.61	.68	.13	.18	.10

NUTS, DRY BASIS

Buckeye, fetid	12.63	6.13	2.48	4.81	73.95	11.44	1.42	3.21	.....	0.11	0.16	0.52
Chestnut, Italian	6.88	3.34	2.42	3.05	84.31	5.69	.38	3.69	0.19	.....	.07	.15
Hazelnut	26.50	61.40	2.16	2.76	7.18	23.88	1.22	3.91	.....	.29	.17	.40
Hickory, shell bark	13.31	74.36	1.51	2.01	8.81	12.13	.74	2.63	.48	(1)	.16	.37
Oak, red	6.56	20.81	3.10	2.42	67.11	6.13	2.99	4.14	9.77	(1)	.07	.14
Oak, rock chestnut	6.94	5.05	2.62	2.22	83.17	6.25	2.50	3.53	10.43	(1)	.09	.15
Oak, scrub	10.25	19.99	3.00	2.12	64.64	9.56	4.00	3.76	11.28	(1)	.14	.19
Oak, scrub chestnut	7.63	6.30	2.42	1.98	81.67	6.88	6.56	3.19	4.43	.07	.08	.15
Oak, white	6.25	6.32	2.47	2.64	82.32	6.00	2.64	3.24	5.58	(1)	.10	.16
Walnut, black	29.25	60.23	1.03	2.76	6.73	27.06	.87	2.01	.25	(1)	.27	.59

TABLE 3.—*Percentage analysis of the fruit and nut products examined*

FRUITS, FRESH BASIS

	Moisture	Crude protein	Ether extract	Crude fiber	Total ash	N-free extract	Available protein	Lignin	Cellulose	Tannin	Calcium	Magnesium	Phosphorus
Apple, narrowleaf crab	87.0	0.75	0.80	2.20	0.41	8.84	0.50	1.55	2.01	0.61	0.01	0.01	0.02
Bittersweet	66.3	5.13	9.69	2.78	1.16	14.94	4.00	3.02	3.44	.45	.09	.10	.12
Blackberry	80.9	1.56	1.45	4.10	.60	11.39	1.25	6.06	2.58	.33	.03	.03	.04
Blackberry, Bailey's	79.6	1.38	1.24	4.93	.88	11.97	1.13	5.84	3.17	.42	.02	.04	.02
Blackhaw	57.1	1.75	5.12	4.41	1.11	30.51	1.56	10.59	3.31	2.55	.02	.03	.06
Blueberry	85.3	.63	.56	1.42	.21	11.88	.50	2.04	1.17	.19	.01	.01	.01
Cherry, wild, whole	64.5	2.38	2.22	7.41	1.01	22.48	2.06	6.63	4.59	.21	.06	.02	.06
Cherry, wild, seeds	11.5	11.56	13.90	45.02	1.39	16.63	9.94	33.90	22.38	------	.16	.07	.17
Cherry, wild, pulp and skin	75.6	1.25	.95	3.25	.77	18.18	1.06	3.35	2.40	.18	.04	.02	.04
Chokeberry, black	75.6	1.25	.84	3.07	.66	18.58	1.00	9.72	2.39	.92	.06	.03	.03
Chokeberry, red	65.6	1.81	1.31	3.18	.87	27.23	1.50	12.43	2.92	2.51	.07	.07	.05
Cucumbertree	73.0	2.00	5.94	7.67	1.32	10.07	1.69	4.48	5.36	.70	.06	.04	.06
Deerberry	83.2	.63	.92	1.88	.27	13.10	.44	1.93	1.44	.29	.01	.01	.01
Dogwood, panicled	57.0	2.94	11.49	11.07	1.45	16.05	2.50	8.86	4.82	.63	.09	.14	.07
Dogwood, red-osier	68.4	2.19	3.79	8.34	1.07	16.21	1.75	8.56	3.79	.50	.09	.06	.07
Elder, American	76.4	2.63	3.06	4.24	1.30	12.37	2.00	3.63	2.49	.64	.03	.05	.08
Grape, frost	69.6	1.63	.26	4.08	.84	23.59	1.25	4.41	2.27	.61	.02	.03	.05
Hackberry	21.4	6.50	3.43	5.57	21.50	41.60	5.56	6.32	4.63	.64	9.76	.38	.17
Hawthorn, cockspur	62.0	1.06	1.25	12.48	1.40	21.81	1.00	7.77	8.86	1.29	.16	.04	.05
Juneberry	80.7	1.56	.86	2.37	.71	13.80	1.25	3.10	2.88	.08	.07	.04	.04
Mountain ash, American	73.7	1.44	1.23	2.11	.82	20.70	1.13	2.52	1.81	1.07	.03	.03	.04
Mountain-holly	59.2	2.88	3.15	7.53	.91	26.33	2.38	7.04	5.91	.39	.05	.05	.05
Nannyberry	53.8	1.94	4.11	3.32	.95	35.88	1.69	14.94	3.09	.73	.05	.02	.06
Spicebush, whole	62.3	4.50	19.14	1.97	2.17	9.92	3.75	1.52	2.26	.51	------	.05	.11
Spicebush, seeds	28.4	13.00	40.26	5.33	1.58	11.43	12.25	3.74	3.51	.42	------	.11	.27
Spicebush, pulp and skin	77.8	1.94	10.62	.90	1.69	7.05	1.38	.76	1.45	.39	------	.03	.06
Sumac, smooth upland	8.1	3.81	10.32	32.07	2.25	43.45	3.25	20.73	25.72	6.33	.15	.06	.15
Sumac, staghorn	8.0	5.00	13.37	27.87	2.77	42.99	4.50	19.92	25.29	4.06	.28	.14	.23
Winterberry, Virginia	68.6	1.94	1.60	4.88	.82	22.16	1.69	2.98	3.96	.21	.04	.06	.03

NUTS, FRESH BASIS

Buckeye, fetid	52.7	6.00	2.90	1.17	2.27	34.96	5.44	0.67	1.52	------	0.05	0.08	0.25
Chestnut, Italian	33.1	4.63	2.23	1.62	2.04	56.38	3.81	.25	2.47	0.13	------	.05	.10
Hazelnut	2.6	25.81	59.80	2.10	2.69	7.00	23.25	1.19	3.81	------	.28	.17	.39
Hickory, shellbark	2.2	13.00	72.72	1.48	1.97	8.63	11.88	.72	2.57	.47	(1)	.16	.36
Oak, red	38.2	4.06	12.87	1.92	1.50	41.45	3.81	1.85	2.56	6.04	(1)	.05	.08
Oak, rock chestnut	50.1	3.44	2.52	1.31	1.11	41.52	3.13	1.25	1.76	5.20	(1)	.04	.08
Oak, scrub	42.0	5.94	11.61	1.74	1.23	37.48	5.56	2.32	2.18	6.55	(1)	.08	.11
Oak, scrub chestnut	44.2	4.25	3.52	1.35	1.11	45.57	3.81	3.66	1.78	2.47	.04	.04	.09
Oak, white	47.3	3.31	3.33	1.30	1.39	43.37	3.13	1.39	1.71	2.94	(1)	.05	.08
Walnut, black	2.9	28.38	58.48	1.00	2.68	6.56	26.25	.84	1.95	.24	(1)	.26	.57

[1] Trace.

The values for nitrogen-free extract are of more indefinite significance than usual because they include the questionable tannin fraction, which reaches a maximum of 11.28 percent in the acorn of the scrub oak.

A lignin determination on the cellulose of the blackhaw revealed that a considerable portion of the lignin had not been removed during the acetic acid-nitric acid digestion. The percent of cellulose in the moisture-free sample was 7.70, and of this total approximately 16 percent appeared as lignin. Whether this means that the acid

digestion is incomplete, or that the lignin value is only an apparent one, remains to be determined.

FRUITS

Unless otherwise stated, the seeds of fleshy fruits were ground in with the remainder. This obviously gives consistent results for the products as used by animals which themselves grind the seeds, but yields improperly high results with animals which do not grind the seeds.

The fruits are predominantly carbohydrate foods; many are high in lignin and cellulose, and some are rich in protein and fat, mainly because of their seeds.

The bittersweet berry consists mainly of thin-walled seeds, with a thin covering of flesh of high moisture content. Its very high content of protein and ether extract, and relatively high content of mineral nutrients, constitute it a decidedly concentrated foodstuff.

The fruits of two species of blackberry, which are of much the same physical character as the bittersweet berry, appear to be of lower nutritive value. The composition, as stated here, represents the value of the blackberry to animals that grind the small, hard seeds and thus expose the kernels to digestion. The blackberry contains appreciable quantities of all the classes of nutrients determined, but is most notable for its high cellulose and lignin content.

The blueberry, deerberry, and juneberry are mainly carbohydrate foods. Aside from their content of nitrogen-free extract they are of moderately low value.

The value of the wild cherry depends largely on whether the shells are broken and the kernels utilized. The pulp and skin are relatively poor in protein and fat, but rich in nitrogen-free extract, while the seeds are moderately rich in protein, decidedly rich in fat, and poor in nitrogen-free extract. The pulp and skin are similar in composition to that of the blueberry, the deerberry, and the juneberry.

The berries of the mountain-ash and the two chokeberries contain nearly 80 percent of nitrogen-free extract, as well as approximately 5 percent each of crude protein and ether extract and therefore must be regarded as decidedly useful winter foods for animals to which they are acceptable. Both of the chokeberries contain high percentages of lignin.

The narrowleaf crab apple also is mainly a carbohydrate food, being relatively poor in protein, ether extract, and mineral nutrients, and rich in nitrogen-free extract. The high content of cellulose, lignin, and tannin are more suggestive of bark than of fruit. The product, however, appears to be characterized by a considerable value for purposes of maintenance.

In spite of its high crude-fiber content the fruit of the cucumbertree appears to be of high nutritive value to those animals that accept it. The ether extract is high, but the dense cellulosic network which lends rigidity to the fruit and its aromatic odor may render the fruit unacceptable to some animals.

The deerberry is a low-protein, low-fat, predominantly carbohydrate food, with no outstanding nutritive peculiarities.

The fruits of dogwoods, especially the panicled dogwood, are high in ether extract, and they have only a moderate content of protein and

mineral nutrients. The crude-fiber content is high, but not so high as to prevent these products from being important winter reserve foods.

The berry of the American elder has a composition that makes it nutritionally superior even to those of the dogwoods. Its lower crude fiber is associated not, as in many products, with a high content of nitrogen-free extract, but with a relatively high content of protein. The ether extract, although not as high as that of the panicled dogwood, is still higher than that of the red-osier dogwood.

The physical characteristics and composition of the frost grape place it in a class with the wild cherry pulp and skin, except that the ether extract is remarkably low. This means that it is mainly a carbohydrate food and therefore of value principally for maintenance purposes. As with other fruits containing a large proportion of seeds to pulp, the value of the frost grape depends largely on the method by which the animal disposes of the seed.

The hackberry is remarkable because of its unusual content of ash, and also of calcium, which, on the dry basis, amounts to 12.42 percent. Magnesium also occurs in an amount greater than in any other fruit or berry. The other constituents are present in moderate quantities, with the exception of lignin and cellulose, which are relatively low. Its magnesium content is twice as high as its phosphorus.

To those animals that can avail themselves of the whole fruit (flesh and seed) the blackhaw is moderately nutritious. It is most notable for its low protein and high lignin and tannin content.

The fruits of the mountain-holly, juneberry, mountain-ash, nannyberry, and winterberry are low-protein, low-fat products with the relatively high carbohydrate content usual among fruits.

The spicebush berry, whether whole or divided between pulp and skin, and seed, is unusual in that it contains approximately 50 percent of ether extract. This extract, however, is highly aromatic and may render the berry unacceptable to some animals. The content of protein is above average, while the percentages of crude fiber and nitrogen-free extract are decidedly low. This berry, therefore, possesses a decidedly high potential nutritive value.

The sumac berries also are rich in ether extract, though not nearly so rich as the berry of the spicebush. Their content of crude fiber is high, and the sum of the lignin and cellulose fractions amounts to approximately 50 percent. The nutritive value of these sumacs is presumably further lowered by their high content of tannin, but a positive qualification of sumac berries is the fact that they are unusually resistant to decay and therefore may serve as important food reserves, especially during the later winter months, when food is scarce.

That sumac berries are efficiently utilized, however, cannot be assumed with confidence, since Errington (*3*) found that not only sumac but also bittersweet berries, rose hips, dried wild grapes and sweetclover seed, individually, failed to maintain captive bobwhites. At the same time it should be realized that maintenance on a single food is a severe test that many useful products cannot satisfy, and that a normally efficient diet might readily be made up of a variety of foods no one of which, by itself, is capable of maintaining the animal consuming it.

The cockspur hawthorn fruit is a decidedly inferior foodstuff. It is deficient in protein and fat, high in crude fiber, and relatively low in nitrogen-free extract. Furthermore, the nitrogen-free extract includes a considerable proportion of tannin.

NUTS

The analyses of all of the nuts represent the kernels only, the hulls being considered as without value.

Nuts in general are characterized by their concentration, as sources of nutriment, but there is much diversity in their content of protein, carbohydrate, and fat. The kernels are all low in lignin and cellulose.

Among the species analyzed, the black walnut, the hickory nut, and the hazelnut are in a class by themselves as high-protein, high-fat, and low-carbohydrate foods, with the hickory nut containing only about half as much protein as the walnut and the hazelnut, but decidedly more fat. The percentage composition of these three nuts is suggestive of that of animal flesh.

The chestnut and the acorns are relatively low in protein, crude fiber, and phosphorus, but contain substantial amounts of available carbohydrate. Three of the five acorns are also low in fat.

The nuts analyzed were all exceedingly low in calcium, the hazelnut being the highest in this constituent, and were also low in phosphorus; but among these species the buckeye, hazelnut, hickory nut, and black walnut were richer in this element than were the chestnut and the acorns. The buckeye was richest of all in ash.

The acorns were all high in tannin content, the red oak, rock chestnut oak, and scrub oak being the highest. The nutritive status of tannin, for wildlife, remains to be determined. Obviously foods which are rich in this constituent are acceptable to many kinds of animals, but not to others.

SUMMARY

Chemical analyses are presented of 35 mast foods, the conventional food analysis being supplemented by determinations of tannin, cellulose, lignin, available protein, calcium, magnesium, and phosphorus.

The sum of the lignin and cellulose fractions was invariably found to be higher than the value determined for crude fiber.

In all products the content of available protein was less than that of crude protein.

The values for nitrogen-free extract of mast foods are of more questionable significance than the corresponding values for foodstuffs in general because they include the tannin fraction, which reached a maximum of 11.28 percent, on the dry basis, in the acorn of the scrub oak.

The fruits and berries are, for the most part, relatively rich in nitrogen-free extract and much less rich in protein and ether extract. They are of only moderate nutritive value, and serve mainly for energy production. The nuts are of greater value, especially because of their content of protein and fat.

In contrast to most fleshy fruits, those of bittersweet, cucumber-tree, panicled dogwood, and spicebush are remarkably rich in ether extract, and are, therefore, relatively concentrated foods.

The hazelnut, hickory nut, and black walnut are very concentrated foods, containing much ether extract and protein, and very little nitrogen-free extract. Among nuts they are relatively rich in phosphorus. Almost all of the nuts analyzed were exceedingly low in calcium, and they usually contained much more magnesium than calcium.

Chestnuts, buckeyes, and most acorns are oil-poor and are relatively rich in nitrogen-free extract; they are not so rich in protein as are the oil-bearing nuts.

The products richest in tannin were the acorns, chokeberries, sumac berries, blackhaw, narrowleafed crab apple, and mountain-ash berries.

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